ADAPTATION DE *STREPTOMYCES SCABIES* AU PÉRIDERME DE LA POMME DE TERRE

par

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SOMMAIRE

Le périderme de la pomme de terre est composé principalement de subérine. La subérine est composée de deux domaines, les domaines polyaliphatique et polyaromatique. Le domaine aliphatique de la subérine est constitué de polyesters d'acides gras avec des acides féruliques estérifiés. L'acide férulique est supposé se lier transversalement à la partie aromatique de la subérine. La subérine aromatique est composée principalement d'acides férulique et coumarique. Des recherches antérieures ont montré l'importance des esters férulés dans le maintien de l'intégrité du périderme, qui agit comme une barrière contre l'entrée de pathogènes. Il est de plus en plus évident que *Streptomyces scabies*, une bactérie causant la gale commune de la pomme de terre, peut dégrader la partie aliphatique de la subérine, mais son aptitude à dégrader le domaine aromatique n'a pas été étudiée.

Dans la première partie de cette thèse, une surexpression hétérologue du gène *sub1* a été faite dans une souche de *E. coli*. Ensuite, la protéine codée par ce gène a été purifiée. L'activité de l'enzyme Sub1 sur divers substrats dont un polymère synthétique (PET) et des biopolymères comme la subérine et la cutine a été testé. Notre étude démontre que Sub1 est bel et bien une cutinase/subérinase puisque des acides gras sont libérés de ces substrats en présence de l'enzyme. Sub1 hydrolysait aussi le polyester synthétique PET et pourrait donc être intéressante dans des applications biotechnologiques.

Dans la deuxième partie, une étude chronologique de la déplétion en acides *trans*-féruliques et en acides *p*-coumariques a été réalisée à l'aide de la chromatographie en phase liquide à haute performance (HPLC) en échantillonnant périodiquement les

surnageants de culture de *S. scabies* souches EF-35 et 87.22 cultivées en présence de ces composés aromatiques. Cette étude a révélé que *S. scabies* peut dégrader les acides *trans*-férulique et *p*-coumarique. Une analyse protéomique de *S. scabies* 87.22 cultivé en présence d'acide *trans*-férulique a permis d'identifier certaines protéines qui pourraient être impliquées dans le catabolisme du ferulate. De plus, l'expression des gènes codant pour les protéines identifiées a été déterminée par qRT-PCR en présence des acides *trans*-férulique et des acides *p*-coumarique. Les gènes testés étaient fortement exprimés en présence des deux composés. Les données suggèrent que le catabolisme des acides *trans*-férulique et des acides *p*-coumarique s'est produit via la voie du β-cétoadipate.

Dans la troisième partie, nous avons comparé les réponses de *S. scabies* 87.22, *S. acidiscabies* ATCC 49003 et *S. turgidiscabies* Car8 lors de leur interaction avec le périderme de la pomme de terre enrichi en subérine. *S. scabies* 87.22 et les espèces émergentes induisant la gale commune (*Streptomyces acidiscabies* ATCC 49003 et *Streptomyces turgidiscabies* Car8) ont été comparées à la fois pour leur capacité à se développer sur le périderme de la pomme de terre enrichi en subérine et à récupérer les nutriments de ce substrat. Les résultats ont révélé que, contrairement à *S. scabies* 87.22, *S. acidiscabies* ATCC 49003 et *S. turgidiscabies* Car8 présentaient une capacité d'utilisation médiocre vis-à-vis des deux constituants principaux de la fraction aromatique de la subérine (acides *trans*-férulique et *p*-coumarique). *S. scabies* 87.22 a également montré une croissance supérieure à celle des autres espèces induisant la gale commune lorsqu'elle était cultivée en culture pure ou en co-culture avec *S. acidiscabies* ATCC 49003 et *S. turgidiscabies* Car8 dans un milieu contenant de la subérine. De plus, *S. scabies* 87.22 semble mieux adapté que les deux espèces de *Streptomyces* à dégrader le matériau cellulosique incrusté dans les parois cellulaires subérisées du périderme de pomme de terre.

Les résultats présentés dans cette thèse visaient à démontrer pour la première fois la capacité de *S. scabies* à dégrader non seulement la partie aliphatique de la subérine, mais également le domaine aromatique. Il a également été démontré que *S. scabies* 87.22 était bien mieux adapté à la plante hôte que les agents pathogènes émergents (*S. acidiscabies* ATCC 49003 et *S. turgidiscabies* Car8). Toutes ces propriétés pourraient conférer un avantage à *S. scabies* 87.22 en tant que saprophyte par rapport aux autres espèces induisant la gale commune et en tant que parasite, car la dégradation de la subérine peut faciliter à la fois la colonisation de la pomme de terre et les processus infectieux.

Mots clés: Sub1, espèces induisant la gale commune, hydroxycinnamates, acide *trans*-férulique, subérine, *Streptomyces scabies*.

SUMMARY

Potato periderm is composed mainly of suberin. Suberin is composed of two domains (polyaliphatic and polyaromatic domains). The aliphatic domain of suberin consists of fatty acid polyesters with esterified ferulic acids. Ferulic acid is believed to cross-link to the aromatic moiety of suberin. The aromatic suberin is composed mainly of ferulic and coumaric acids. Previous research reported the importance of ferulate esters in maintaining the integrity of the periderm which acts as a barrier against pathogens entry. Evidence accumulates that *Streptomyces scabies*, a potato common scab-inducing bacterium, can degrade the aliphatic part of suberin but its ability to degrade the aromatic domain has not been studied.

In the first part of this thesis, a heterologous overexpression of the sub1 gene was made in an *E. coli* strain. Then, the protein encoded by this gene was purified. The activity of the enzyme Sub1 on various substrates including the synthetic polymer polyethylene terephthalate (PET) and the natural polymers such as suberin and cutin has been tested. Our study demonstrates that Sub1 is a cutinase/suberinase since fatty acids are released from these substrates in the presence of this enzyme. Sub1 also hydrolyzed the synthetic polyester polyethylene terephthalate (PET) and could therefore be of interest in biotechnological applications.

In the second part, a time course study of both *trans*-ferulic and *p*-coumaric acids depletion was carried out using High Performance Liquid Chromatography (HPLC) by periodically sampling culture supernatants of *S. scabies* strains EF-35 and 87.22 grown in the presence of these aromatic compounds. This study revealed that *S. scabies* can degrade both *trans*-ferulic and *p*-coumaric acids. A proteomic analysis of

S. scabies 87.22 in the presence of *trans*-ferulic acid allowed the identification of some proteins which could be involved in the catabolism of ferulate. Moreover, the expression of the genes encoding the identified proteins was determined by qRT-PCR in the presence of both *trans*-ferulic and *p*-coumaric acids. The tested genes were highly expressed in the presence of both compounds. The data suggest that *trans*-ferulic and *p*-coumaric acids catabolism occurred via the β-ketoadipate pathway.

In the third part, we compared *S. scabies* 87.22, *Streptomyces acidiscabies* ATCC 49003 and *Streptomyces turgidiscabies* Car8 in their interaction with suberin-enriched potato periderm. *S. scabies* 87.22 and the emerging common scab-inducing species (*Streptomyces acidiscabies* ATCC 49003 and *Streptomyces turgidiscabies* Car8) were compared for both their ability to grow on suberin-enriched potato periderm and their retrieval of nutrients from this substrate. Results revealed that, in contrast to *S. scabies* 87.22, the other two common scab-inducing species *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 exhibited poor utilization of the two main constituents of the aromatic moiety of suberin (*trans*-ferulic and *p*-coumaric acids). *S. scabies* 87.22 also showed higher growth rates than the other common scab-inducing species when grown in pure culture, or in co-culture, with *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 in suberin-containing medium. Furthermore, this study also demonstrated that *S. scabies* 87.22 is better adapted than the two other *Streptomyces* species to degrade the cellulosic material embedded in the suberized cell walls of potato periderm.

The results presented in this thesis demonstrated for the first time the ability of *S. scabies* to degrade not only the aliphatic part of suberin but also the aromatic domain. *S. scabies* was shown also to be better adapted to its host plant compared to the emerging pathogens *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8. All

these properties could give an advantage to *S. scabies* 87.22 over the other common scab-inducing species as both a saprophyte and as a parasite since suberin degradation may facilitate both the potato tuber colonization and the infection processes.

Key words: Sub1, common scab-inducing species, hydroxycinnamates, *trans*-ferulic acid, suberin, *Streptomyces scabies*.

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
µg	Microgram
µL	Microliter
µm	Micrometer
µM	Micromolar
BLAST	Basic Local Alignment Search Tool
C	Cytosine
cDNA	Complementary Deoxyribonucleic acid
Ceb	Cellobiose binding protein
CebR	Cellulose utilization repressor
cfu	Colony-forming unit
CM	Control medium
CM+C	Control medium supplemented with *p*-coumaric acid
CM+F	Control medium supplemented with *trans*-ferulic acid
CM+S	Control medium supplemented with suberin
COG	Clusters of Orthologous Groups of proteins
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
Da	Dalton
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
Fae	Feruloyl esterases
FDR	False discovery rate
g	Gram

G	Guanine
h	Hour
HPLC	High Performance Liquid Chromatography
IAA	Indole-3-acetic acid
IaaH	Indole-3-acetamide hydrolase
IaaM	Tryptophan monooxygenase
IAM	Indole-3acetamide
IPTG	Isopropyl β-D-1-thiogalactopyranoside
kb	Kilobase
KEGG	Kyoto Encyclopedia of Genes and Genomes
kg	Kilograms
kV	Kilovoltage
L	Liter
LSD	Least Significant Difference
M	Mega
m/z	Mass-to-charge ratio
min	Minutes
mL	Milliliter
mmol	Millimoles
MS	Mass spectrometer
NCBI	National Center for Biotechnology Information
ng	Nanogram
NPB	Nitrophenyl butyrate
nm	Nanometer
NOS	Nitric oxide synthetase
NSAF	Normalized Spectral abundance factor
NSpC	Normalized Spectral Count
PAHs	Polycyclic aromatic hydrocarbons
PBS	Phosphate buffered saline

PCR	Polymerase Chain Reaction
PET	Polyethylene terephthalate
pH	Potential hydrogen
PW	Primary wall
qRT-PCR	Quantitative real time polymerase chain reaction
Rf	Retention factor
RNA	Ribonucleic acid
rpm	Rotations per minute
s	Second
SD	Standard deviation
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide gel electrophoresis
SpC	Number of Spectral counts
SW	Secondary wall
TA	Terephthalic acid
TLC	Thin layer chromatography
TW	Tertiary wall
v/v	Volume per volume
V_{max}	The maximal velocity
w/v	Weight per volume
YME	Yeast Malt Extract

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CHAPTER 1

GENERAL INTRODUCTION

1.1. The potato

The potato plant, *Solanum tuberosum* L., is an annual perennial plant belonging to the Solanaceae family. The potato is characterized by its tubers which are very rich in starch. The potato originates from the Andes region of South America and was introduced to Europe by Spaniards in the 16th century and was subsequently distributed all over the world (Brown, 1993). The outbreak of late blight in Ireland in 1840 caused a great famine and led to significant emigration to Canada and the United States. The potato is one of the most important food crops around the world and is grown in more than 150 countries. The potato comes in the fourth position of the most important food crops after maize, wheat and rice (Ezekiel *et al*., 2013). Potato tubers are rich in carbohydrates, vitamins, minerals, fibers and proteins and can be transformed into several products, such as chips, french fries, flours, etc.

1.2. The potato in Canada

Canada is the 13th largest potato producing country in the world and the second in the Americas (after the United States). In Canada, potato farmers planted 347,416 acres (140, 594 hectares) of potato in 2018. Potato is grown in all canadian provinces but mostly in Prince Edward Island, New Brunswick, Manitoba, Alberta and British Columbia. Prince Edward Island accounted for 24.2% of the total potato planted area in 2018, followed by Manitoba (18.5%) and Alberta (15.9%) (Government of Canada,

2018). In 2017, at 57.9 kg/person per year, potatoes accounted for 35% of all vegetables consumption in Canada and were far ahead of the tomato (24.8 kg/person, 15%) (Ministry of Agriculture, Fisheries and Food, Québec). Québec is the 5th largest potato producing province in Canada. In 2017, more than 600 companies in Québec produced 590,000 tonnes of potato on an area of 17,400 hectares, generating sales of $173 million (Ministry of Agriculture, Fisheries and Food, Québec).

1.3. Potato tubers

The underground part of the potato plant has fibrous roots and stolons (underground stems). The fibrous roots allow the plant to withdraw the essential elements and nutrients from the soil for its growth. Stolons act as the plant reserve organ carrying the tubers at their ends. Potato tubers represent about 75 to 85% of the total dry matter of the plant. An early event in potato tuber formation is the replacement of the epidermis of the expanding stolon tip by periderm. The periderm originates from cell division in the epidermis and hypodermis. The periderm is composed of three types of tissues; phellem, phellogen and phelloderm (Figure 1.1). The phellogen layer in the periderm form several layers of suberized cells (phellem) to the outside and the phelloderm to the inside. The phellem cells are rectangular and embedded with suberin to form a protective layer which is impermeable to water and gases and resists infection by micro-organisms (Tyner *et al*., 1997) (Figure 1.1). Lenticels are formed in the periderm from divisions of cells below the stomata of the original epidermis. These divisions form a tissue of rounded cells with intercellular spaces opening to the exterior to allow the gas exchange through periderm (Tyner *et al*., 1997). These lenticels can act as an entrance for several plant pathogens (Scott *et al*., 1996).

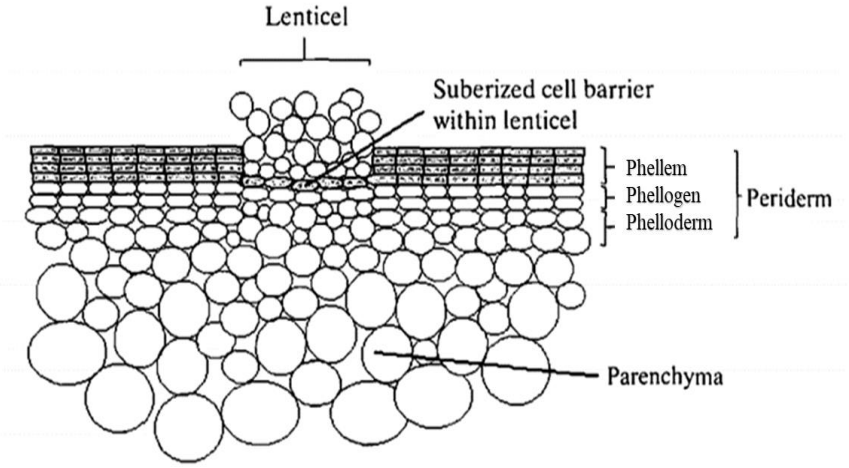


Figure 1.1. Diagrammatic representation of a cross section through a potato tuber (Tyner *et al*., 1997).

1.4. Potato tuber cells

Like all plant cells, the cell wall of potato cells is composed of three parts: 1) the middle lamella, which is the outer cell wall layer consisting mainly of polysaccharides called pectins (Jarrige *et al*., 1995), 2) the primary cell wall, composed of cellulose microfibrils contained within a gel-like matrix of hemicellulose fibers and pectin polysaccharides, and 3) the secondary cell wall, a layer formed between the primary cell wall and the plasma membrane. Once cells have stopped dividing and growing, the primary cell wall may thicken to form a secondary cell wall. This rigid layer is composed of cellulose and hemicellulose to strengthen and supports the cell and can be enriched in phenolic compounds (Figure 1.2). Potato cells contain amyloplasts instead of chloroplasts which are specialized in starch storage.

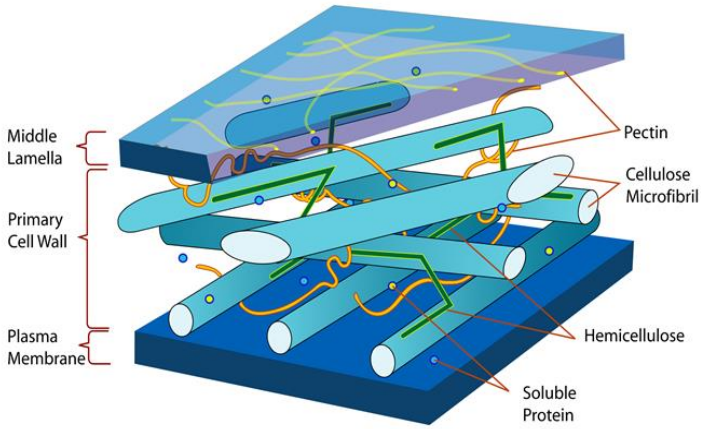


Figure 1.2. Plant cell wall structure.

(https://www.thoughtco.com/cell-wall-373613)

The cell walls of potato cells, especially those located at the surface of tubers, act as the first barrier to prevent the pathogens entry. As most cells, phellem cells possess a primary cell wall, a secondary cell wall but also a tertiary wall (the innermost layer of the secondary wall) (Figure 1.3) (Beaulieu *et al*., 2016). When the cells of the phellem develop, they will become suberized and die (because the suberin deposited in the cells prevents any cellular exchange), to form a protective layer (Sabba and Lulai, 2002) against pathogens entry. Suberin found in phellem cells can play a protective role against pathogens (Kolattukudy, 1984; Lulai and Corsini, 1998) by forming a biochemical barrier due to the lipidic and phenolic compounds found in the polymer (Kolattukudy, 1984). The periderm becomes mature with complete lipid coverage two or three weeks after harvest of the potatoes (Neubauer *et al*., 2013).

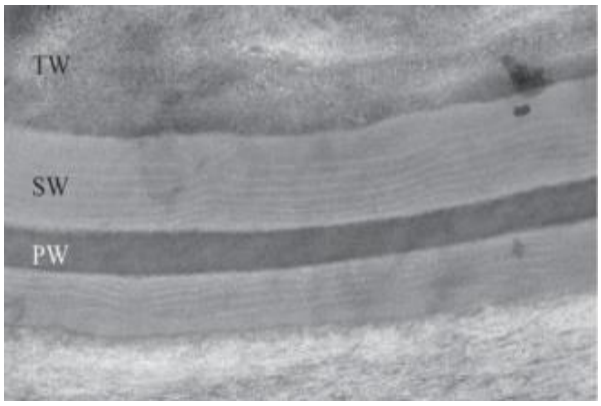


Figure 1.3. Cell wall structure of the potato phellem, showing suberized secondary wall (SW), primary wall (PW) and tertiary wall (TW) (Beaulieu *et al*., 2016).

1.5. Chemical composition of suberin

Suberin is a waxy substance with a complex structure. It is composed mainly of two domains, a glycerol-based polyaliphatic domain located between the primary cell wall and the plasma membrane which is covalently linked to a hydroxycinnamic acid-monolignol polyphenolic domain embedded in the primary cell wall (Figure 1.4) (Bernards, 2002).

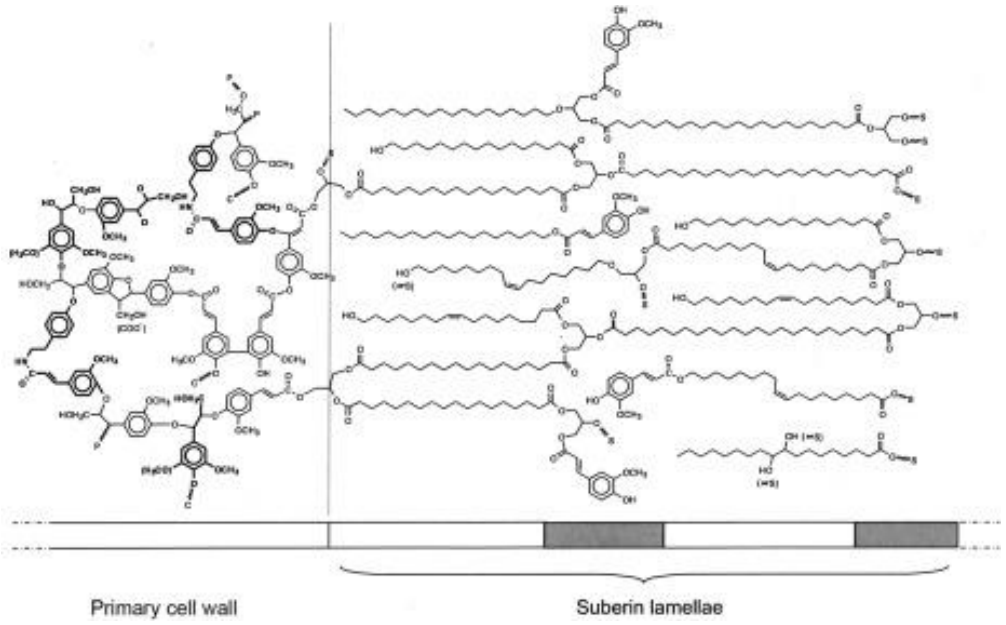


Figure 1.4. Tentative model for the structure of potato suberin (Bernards, 2002).

The structure of the aliphatic part of suberin was elucidated based on the analysis of the degradation products obtained by depolymerization through cleavage of the ester bonds using the alkaline hydrolysis or methanolysis (Gandini *et al*., 2006). The obtained monomers of the aliphatic moiety of suberin are mainly long chains of aliphatic dicarboxylic acids (16-24 carbon atoms), ω-hydroxy fatty acids (from 20 to 30 carbon atoms), very long chain fatty acids (≥ 30 carbon atoms), fatty alcohols and glycerols (Bernards 2002; Gandini *et al*., 2006) (Figure 1.5). The composition of suberin varies according to the plant species and tissues (Graça and Santos, 2007). Cutin, which is another plant polymer and the main component of the plant cuticle, covers all aerial surfaces of plant. It is very similar to the aliphatic domain of suberin. However, unlike suberin, cutin rarely contains molecules with chains containing more than 20 carbon atoms (Beisson *et al*., 2012).

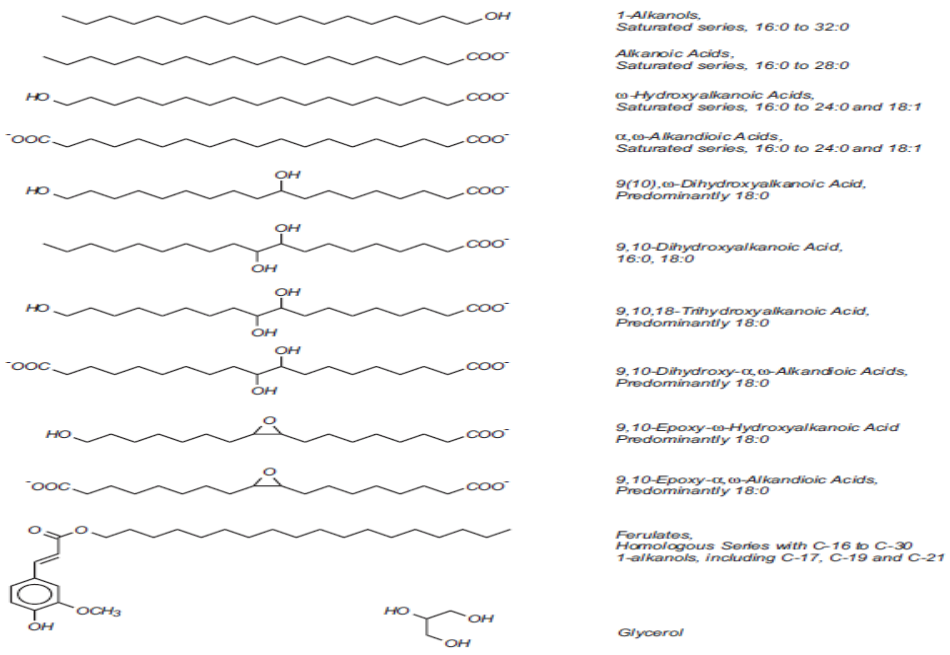


Figure 1.5. Aliphatic precursors of suberized tissues (Bernards, 2002).

The polyaromatic domain of suberin is similar to lignin (Bernards, 2002). Upon depolymerization of suberin, a mixture of hydroxycinnamic acids (particularly ferulic acid) is released (Figure 1.6). Although both suberin and lignin contain monolignols, certain hydroxycinnamic acids and their derivatives, such as feruloyltyramine, are found only in suberin (Bernards *et al*., 1995).

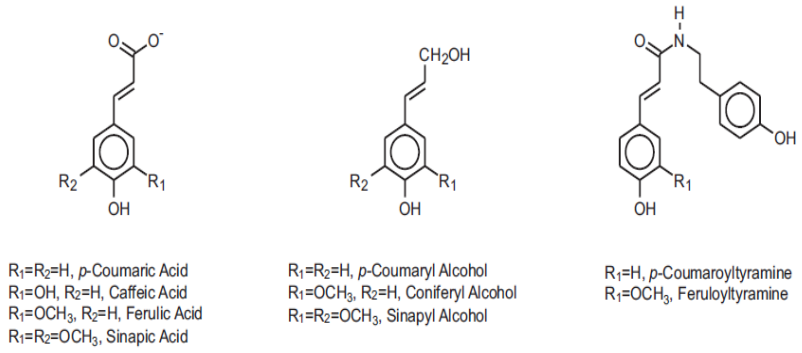


Figure 1.6. Phenolic precursors of suberized tissues (Bernards, 2002).

Serra *et al*. (2010) suggested that ferulic acid plays an important role in the crosslinking of the two suberin domains. When the gene encoding a fatty omega-hydroxyacid/fatty alcohol hydroxycinnamoyl transferase (FHT) was silenced in potato, the resulted tubers contained much smaller amounts of ferulate esters. FHT enzyme is known to conjugate ferulic acid with omega-hydroxyacid and fatty alcohols. Moreover, the maturation was prevented, and the water loss increased greatly in the potato tubers with the knocked out gene. This suggests that ferulic acids play important role in periderm integrity (Serra *et al*., 2010).

1.6. Common scab of potato

Potato diseases can reduce the yield, quality and marketability of the tubers. Potato can be infected by different pathogens including fungi, oomycetes, bacteria, viroids, viruses, protozoa and nematodes (Hooker, 1981) and these pathogens can affect the plant during all stages of development, and even during storage.

Common scab is a potato disease characterized by superficial or deep corky lesions on tubers (Lerat *et al*., 2009) (Figure 1.7) or on the roots of various vegetables such as beet, radish, and parsnip (Goyer and Beaulieu, 1997). The size and color of the common scab lesions are quite variable (Beauséjour *et al*., 2003). Although the disease does not affect crop yield (unless in severe cases) and the infected tubers are safe for human consumption, these infections reduce the marketability of infected tubers causing significant economic losses. For example, in Canada, the total economic loss from potato common scab was estimated to be between 15.3 to 17.3 million Canadian dollars for the year 2002 (Hill and Lazarovits, 2005). Common scab of potato generates economic losses of about 15% in Quebec (Hill and Lazarovits, 2005).

Figure 1.7. Lesions of common scab on potato tubers (Lerat *et al*., 2009).

1.6.1. The main common scab-inducing species

Common scab is caused by pathogenic streptomycetes including *Streptomyces scabies* (*Streptomyces scabiei*), *Streptomyces acidiscabies*, *Streptomyces turgidiscabies*, *Streptomyces stelliscabiei* and *Streptomyces europaeiscabiei* (Healy *et al*., 2000; Loria *et al*., 1997; Miyajima *et al*., 1998; Pasco *et al*., 2005). Pathogenic streptomycetes acquire their pathogenicity through the presence of pathogenicity islands (PAIs) on their chromosomes. These islands encode different virulence factors and are absent from non-pathogenic strains of the same or closely related species (Gal-Mor and Finlay, 2006).

Streptomycetes produce about 70% of clinically useful antibiotics discovered to date (Bérdy, 2005). They are responsible for the characteristic smell of soil, which is related to the production of the volatile compound, geosmin (Bear and Thomas, 1964). The genus *Streptomyces* is a Gram-positive bacterium, strictly aerobic and belongs to the order Actinobacteria. Streptomycetes have a high guanine and cytosine (G+C) content and can undergo a complex morphological and physiological differentiation process during their life cycles (Burger and Eichenlaub, 2003). *Streptomyces* live in

soil mostly as saprophytes and produce extracellular hydrolytic enzymes. These extracellular enzymes break down structural polymers such as lignin and cellulose to provide nutrients (Crawford and Crawford, 1980). *Streptomyces* (Figure 1.8) form sporogenic aerial hyphae (Stackebrandt *et al*., 1997) and its spores are resistant to desiccation and can germinate and develop into branched filamentous hyphae when conditions become favorable. Streptomycetes play an important role in the decomposition and mineralization of organic matter through the production of extracellular enzymes, thus bringing an important contribution to the carbon cycle and nutrient recycling in the environment (Lewin *et al*., 2016).

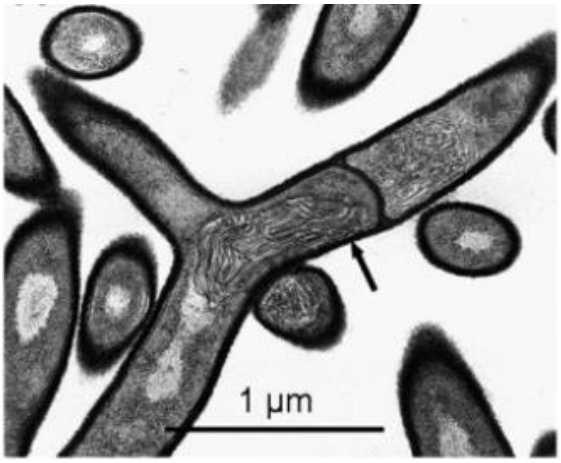


Figure 1.8. Electron micrograph of *Streptomyces scabies* EF-35 (Lerat *et al*., 2012).

1.6.2. *Streptomyces scabies*

Streptomyces scabies is the main causal agent of potato scab (Goyer *et al*., 1996). It was first described by Thaxter in 1890, but a new description of the species was later reported by Lambert and Loria (1989b). It is known for its ability to produce a melanoid pigment. The species is characterized by its smooth, grey spores which are carried on

spiral chains. These spiral chains are mycelial filaments approximately 1 µm in diameter which carry spores that are between 0.5 and 1 µm in diameter (Figure 1.9). *S. scabies* growth is inhibited on solid medium when the pH is below 5 (Lambert and Loria, 1989b). The fully sequenced genome of *S. scabies* strain 87.22 is composed of approximately 10.1 M base pairs with a G+C content of 71.45%.

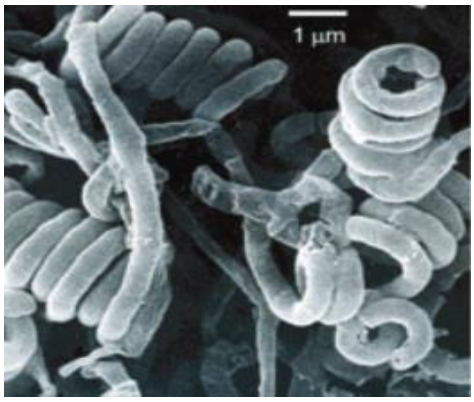


Figure 1.9. Electron micrograph of filamentous mycelium of *Streptomyces scabies* (Lerat *et al*., 2009).

1.6.2.1. Disease cycle of *Streptomyces scabies*

S. scabies can survive during winter in soil and on the surface of tubers and decaying plant material. In the spring, the pathogen is spread from one location to another by splashing water (irrigation or rain) and wind, and on seed tubers and farm equipment with leftover soil residue. When the spores encounter the potato, they will germinate, and the infection process begins. The pathogen invades the periderm of the tuber by entering mainly by lenticels or wounds (Adams and Lapwood, 1978), during the first five weeks of the development of tubers. Direct penetration has also been reported (Loria *et al*., 2003). After penetration, the pathogen can grow through up to three

peridermal cell layers, causing the death of these cells. The bacterium then feeds on these cells saprophytically. The pathogen also secretes a toxic substance (thaxtomin). This causes the tuber cells to divide rapidly leading to suberization of cells which will be converted into cork (suberized) cells that isolate the bacterium and the surrounding tuber cells. As the tuber cells above this suberized layer die, the pathogen feeds on them. As the suberized layers are pushed out and sloughed off, the pathogen grows and multiplies in the additional dead cells. This growth cycle may occur several times throughout the growing season, enlarging the lesion. Lesion size will also vary depending on when infection occurs. Generally, the earlier a tuber becomes infected, the larger the lesion. Figure 1.10 summarizes the disease cycle of the disease (Agrios, 2005).

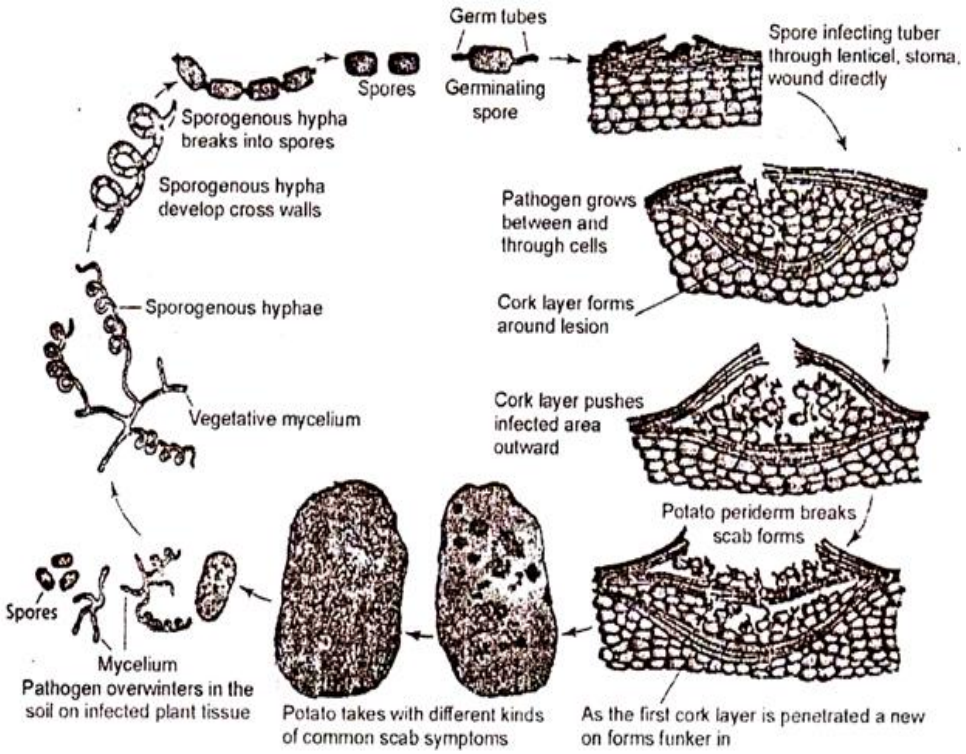


Figure 1.10. Disease cycle of potato common scab caused by *S. scabies* (Agrios, 2005).

1.6.2.2. Virulence factors of *S. scabies*

1.6.2.2.1. Factors involved in external colonization

Pathogenicity is defined as ability of an organism to cause disease or damage to its host while virulence refers to the degree of pathology caused by the organism. Successful colonization of a host plant requires that the pathogen has effective mechanisms for penetrating the plant tissues. *S. scabies* can infect tissues of potato tuber and develops intercellularly after its entry through lenticels or wounds (Agrios, 2005). *S. scabies* can also use the direct penetration through the mechanical force (Figure 1.11A) (Loria *et al*., 2003). Thaxtomin produced by plant pathogenic streptomycetes was also shown to aid penetration of developing plant tissues by inhibiting primary cell wall development (Loria *et al*., 2003), therefore contributing to virulence. The filamentous nature of *S. scabies* allows it to colonize aggressively the tuber surface of the potato. Hydrophobin-like compounds are thought to play a role in host-pathogen interactions since an attachment matrix at the interface of potato tuber cells and *S. scabies* hyphae colonizing those cells has been detected (Figure 1.11B) (Loria *et al*., 2003). Hydrophobins are small, secreted hydrophobic proteins that contribute to the pathogenicity of some fungi. The direct penetration is likely to be accompanied by secretion of hydrolytic enzymes (Komeil *et al*., 2014) that would facilitate the breakdown of the surficial barrier and the degradation of walls, as suggested by microscopic observations of infected tissue (Błaszczak *et al*., 2005).

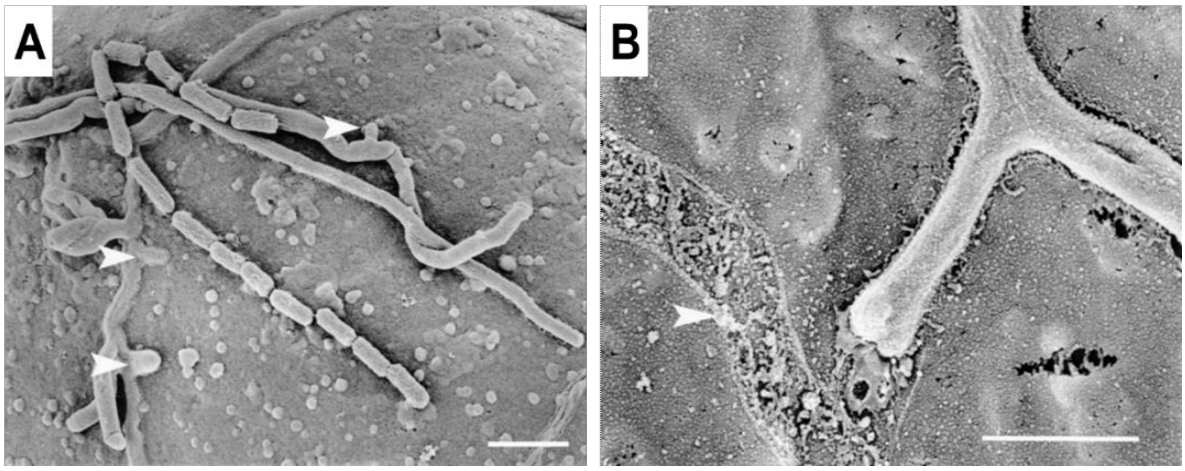


Figure 1.11. Colonization and penetration of potato by *S. scabies*.

(A) *S. scabies* directly penetrates the cells of potato by using short branches emerging from the main hyphae. The short hyphae are perpendicular to the primary hyphae and penetrate within a very short distance from the branch point, indicating that these secondary hyphae are in fact penetration structures (arrow heads). (B) an attachment matrix at the interface of potato tuber cells and colonizing hyphae of *S. scabies*. The arrow shows a track left by the removal of a *Streptomyces* hypha, suggesting bacterial attachment (Loria *et al*., 2003).

1.6.2.2.2. Factors involved in internal colonization

1.6.2.2.2.1. *nec1* gene

The *nec1* gene is responsible for the synthesis of a necrosis factor (Bukhalid and Loria, 1997). *S. scabies,* as most potato common scab-inducing species, possess *nec1* (Bukhalid *et al*., 1998). When a fragment of 9.4 kb containing the *nec1* region was transferred to *S. lividans*, a non-pathogenic *Streptomyces* species, the

recombinant strain was able to cause necrosis and colonization of the potato slices (Bukhalid and Loria, 1997). It was proposed that *nec1* protein is a virulence factor which is secreted at the beginning of the infection (Joshi *et al*., 2007a). However, *nec1* is not necessary for pathogenicity. It would be useful for the colonization of the host plant or for the suppression of defense responses of the host plant. Radish plants inoculated with the *S. turgidiscabies* mutant ∆ *nec1* are still infected, but the mutant failed to colonize the meristem of radish root (Joshi *et al*., 2007a).

1.6.2.2.2.2. *tomA* gene

The *tomA* gene is an orthologous gene to genes encoding tomatinases in pathogenic fungi of tomato. This gene was first discovered in the pathogenicity island of *S. turgidiscabies* Car8 (Kers *et al*., 2005) but was also identified in the genome of *S. scabies* 87.22 (Seipke and Loria, 2008). Tomatinases belong to a class of secreted enzymes, called saponinases (Kers *et al*., 2005). Saponinases are known to detoxify the phytoanticipins which are antimicrobial compounds produced by plants to defend themselves against pathogens (Bouarab *et al*., 2002). The severities of disease for tomato seedlings affected by *S. scabies* 87.22 wild-type and ∆ *tomA* strains were indistinguishable, suggesting that tomatinase is not important in pathogenicity on tomato plants. However, presence of *tomA* in several pathogenic species of *Streptomyces* suggests their involvement in the suppression of plant defense mechanisms to facilitate internal colonization (Seipke and Loria, 2008).

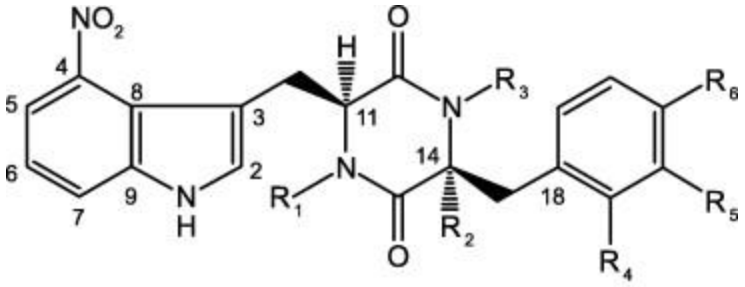
1.6.2.2.2.3. Indole acetic acid

The ability of *S. scabies* to produce indole-3-acetic acid (IAA), a plant auxin, is well known (Manulis *et al*., 1994). *S. scabies* was found to produce indole-3-acetic acid (IAA) through the Indole-3-acetamide (IAM) pathway. Two enzymes, tryptophan monoxygenase (IaaM) and indole-3-acetamide hydrolase (IaaH), participate in IAA synthesis via the IAM pathway. The orthologous of those two genes were identified in the genome of *S. scabies* 87.22 based on amino acid sequences homology. The identified genes in *S. scabies* were named as (SCAB_75511) for tryptophan monoxygenase and (SCAB_75501) for indole-3-acetamide hydrolase (patten *et al*., 2013). When deletion mutants of these genes were generated, the IAA production by all the three generated mutant strains was lower than the wild type strain (87.22) (Hsu, 2010). When inoculated onto radish seedlings, all three mutant strains were reduced in virulence relative to the wild type strain. Genetic complementation of the deletion strains partially restored both IAA production and virulence on radish seedlings suggesting that both (SCAB75511) and (SCAB75501) genes contribute to the virulence of *S. scabies* through the synthesis of IAA which is considered as a virulence factor (Hsu, 2010). The exact role of IAA in *S. scabies* pathogenicity is unknown but as in *Pseudomonas syringae,* IAA is believed to stimulate the release of saccharides from the wall of plant cells, which could provide a source of nutrients for microorganisms and facilitate pathogens colonization (Bender *et al*., 1999).

1.6.2.2.3. Toxins

1.6.2.2.3.1. Thaxtomins

All common scab-inducing species produce a family of phytotoxic secondary metabolites known as thaxtomins. The thaxtomins are cyclic dipeptides (2,5-diketopiperazines) formed from the condensation of two aromatic amino acids, L-phenylalanine and L-tryptophan containing a 4-nitroindole group which is essential for the phytotoxicity of these compounds (King and Calhoun, 2009). Eleven members of this family have been isolated and characterized (Figure 1.12). These members can be distinguished by the presence or absence of N-methyl or hydroxyl groups on the basic structure. When the 4-nitro group is either removed, or shifted to the 5, 6 or 7 positions of the indole ring; or if the phenyl side chain is replaced; or conversion to the D,L configuration instead of the L,L configuration of the compound will result in a total loss of compound toxicity (King *et al*., 1992).



Compound	R_1	R_2	R_3	R_4	R_5	R_6
1	Me	OH	Me	H	OH	H
2	Me	OH	Me	OH	H	H
3	Me	H	H	H	H	H
4	Me	OH	Me	H	H	H
5	Me	H	Me	H	H	H
6	Me	OH	H	H	H	H
7	Me	OH	Me	H	H	OH
8	Me	OH	Me	H	OH	OH
9	Me	OH	H	H	OH	H
10	H	OH	Me	H	OH	H
11	H	H	H	H	H	H

Figure 1.12. Structural formula of the thaxtomins produced by phytopathogenic species of *Streptomyces*. Compound 1 represents thaxtomin A and compound 4 corresponds to thaxtomin B (King and Calhoun, 2009).

Thaxtomin A is the main phytotoxin produced by pathogenic *S.* scabies (Healy *et al*., 2000). Thaxtomins are yellowish secondary metabolites secreted during the transition from vegetative phase to stationary phase. Thaxtomins induce phenotypic changes in the host plant, such as cell hypertrophy, stunted and seedling growth retardation (Leiner *et al*., 1996), tissue necrosis and inhibition of cellulose synthesis (Scheible *et al*., 2003), alteration in the influx of Ca^{2+} (Errakhi *et al*., 2008) and H^+ ions (Tegg *et al*., 2005) in plant cells and programmed cell death (Duval *et al*., 2005).

The genes needed to synthesize thaxtomins are present on a single locus (*txt*) on the chromosome of the pathogenic species (Figure 1.13A). Two non-ribosomal peptide synthetases, TxtA and TxtB, are required to form the cyclic diketopiperizine moiety from the amino acids phenylalanine and tryptophan (Healy *et al*., 2000). Nitric oxide synthetase, TxtD/NOS, is responsible for the nitration of the indole group of L-tryptophan (Johnson *et al*., 2009; Kers *et al*., 2004). The *txtD* gene encodes a nitric oxide synthetase (NOS) that converts L-arginine to nitric oxide (Figure 1.13B). The deletion of the *txtD* gene in *S. turgidiscabies* drastically decreases the production of thaxtomin A (Barry *et al*., 2012). In addition, another enzyme appears to be involved in the nitration reaction, cytochrome P450 monooxygenase, encoded by the *txtE* gene (Bignell *et al*., 2010). Following cyclization, the compound is hydroxylated by cytochrome P450 monooxygenase (TxtC) (Healy *et al*., 2000). Sequence analysis of the thaxtomin biosynthetic cluster in *S. acidiscabies* previously revealed the presence of a small open reading frame (designated ORFX) that is located immediately downstream of the *txtB* gene (Healy *et al*., 2000). This gene is conserved in the *S. turgidiscabies* and *S. scabies* thaxtomin biosynthetic clusters and is predicted to encode a protein belonging to the MbtH-like superfamily. This family, named after the MbtH protein from the mycobactin biosynthetic cluster in *Mycobacterium tuberculosis*, consists of small proteins (about 70 amino acids) frequently associated with the gene clusters for non-ribosomal biosynthesis of peptide antibiotics and siderophores (Baltz, 2011). Although the function of these proteins is unknown, recent studies suggest that they are required for the biosynthesis of some non-ribosomally-synthesized secondary metabolites. Transcriptional analysis of the mbtH-like gene in the *S. scabies* thaxtomin biosynthetic cluster (*txtH*; Figure 1.13A) indicated that the gene is expressed under thaxtomin-inducing conditions and that expression is dependent on *txtR* (Figure 1.13A) (Bignell *et al*., 2010).

Thaxtomin is produced in vitro only when pathogenic bacteria grow in presence of plant extracts (Beauséjour *et al*., 1999). In vitro, biosynthesis of the toxin occurs during secondary metabolism (Lerat *et al*., 2010) and is favored by the presence of cellobiose, a disaccharide derived from the degradation of cellulose. TxtR is an AraC/XylS family protein regulated by cellobiose and transcription of *txtR* and thaxtomin biosynthetic genes was upregulated in response to cellobiose (positive transcription regulator) (Joshi *et al*., 2007b).

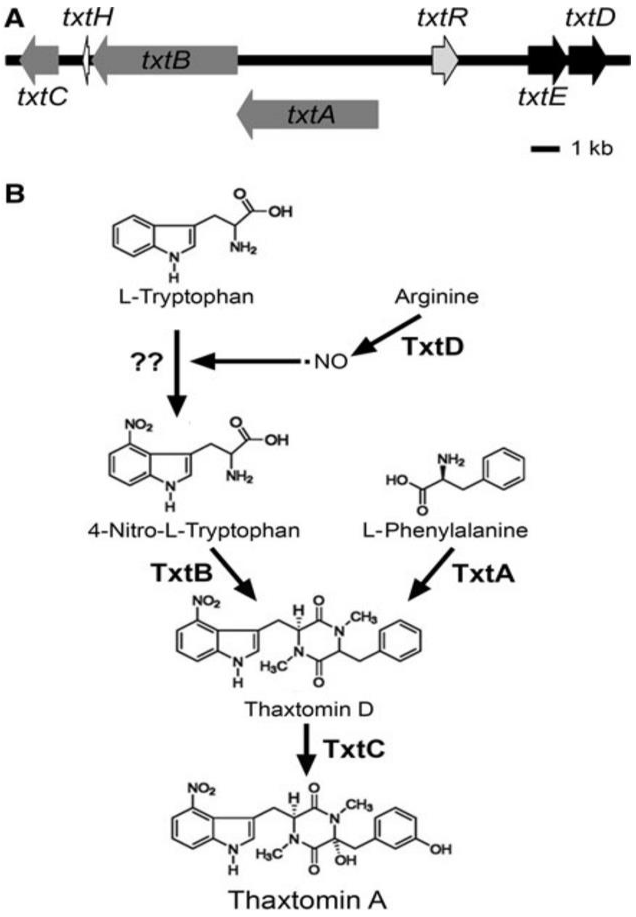


Figure 1.13. (A) thaxtomin biosynthetic gene cluster in *S. scabies* 87.22. (B) thaxtomin A biosynthetic pathway in *Streptomyces* (Bignell *et al*., 2010).

A more recent study identified cellulose utilization repressor (CebR) in the thaxtomin biosynthetic cluster (Francis *et al*., 2015). CebR is known to be involved in the primary metabolism and nutritional functions in non-pathogenic streptomycetes. However, In *S. scabies*, cellobiose and cellotriose inhibited the ability of CebR to bind to DNA, leading to an increased expression of the thaxtomin biosynthetic and regulatory genes *txtA*, *txtB*, and *txtR*. Deletion of CebR results in constitutive thaxtomin A production and hypervirulence of *S. scabies*. Thus, the pathogenicity of *S. scabies* is under dual control, under the control of CebR and TxtR proteins. CebR is a cellobiose sensor, and the key to block the expression of the *txtR* gene. On the other hand, the *txtR* gene is key to unlock the production of thaxtomin (Figure 1.14) (Francis *et al*., 2015). CebR-binding sites also found in *S. acidiscabies* and *S. turgidiscabies* suggesting that CebR is most likely an important regulator of virulence in these species as well.

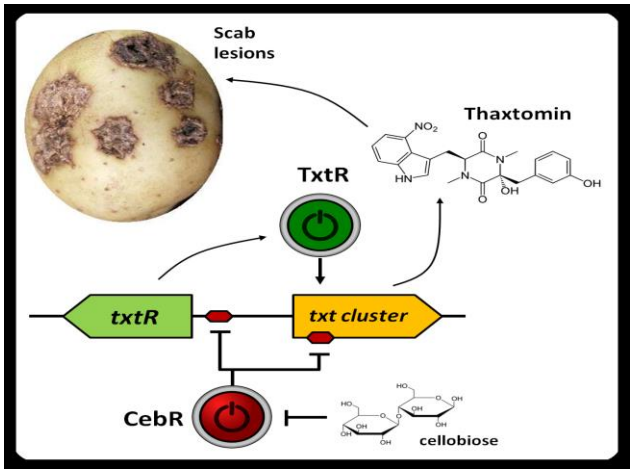


Figure 1.14. Involvement of CebR in the control of expression of the thaxtomin biosynthetic and regulatory genes.

(http://www.reflexions.uliege.be/cms/c_386474/en/thaxtomin-a-next-generation-weed-killer?portal=j_55&printView=true)

Cellobiose was identified as an inducer of thaxtomin A synthesis in *S. scabies* (Johnson *et al*., 2007). However, when the bacterium was grown in the presence of cellobiose as the sole carbon source, there was a weak induction of *txt* genes

expression which are responsible for the production of thaxtomins. Similarly, the addition of suberin to minimal medium induced a low production of thaxtomin A (Beauséjour *et al*., 1999). However, when suberin and cellobiose are combined in the culture medium, the synthesis of thaxtomin A increased considerably and the expression of the thaxtomin biosynthetic genes (*txtA*, *txtB*, *txtD*/*nos* and *txtC*) increased significantly. This suggests that *S. scabies* needs both molecules to maximize thaxtomins production. Therefore, it is possible that the ability of *S. scabies* to produce glycosyl hydrolases, including cellulases, is correlated with the pathogenicity of this bacterium, because it is closely related to the degradation of cellulose to cellobiose.

Padilla-Reynaud *et al*. (2015) proposed a model that could explain the effect of suberin and cellobiose on thaxtomin production in *S. scabies*. In this model, the constituents of the potato periderm promote the onset of *S. scabies*' virulence mechanisms specifically, the major periderm constituents, cellulose and suberin, both play a role in triggering the production of thaxtomins. In the presence of cellulose only (Figure 1.15A), a low amount of cellulases are produced allowing the release of cellobiose, the inducer of thaxtomin biosynthetic genes. However, cellobiose was shown to lock morphogenesis and secondary metabolism (Lerat *et al*., 2010) by allowing the production of a subtilase-like protease inhibitor and, thus, limiting the production of secondary metabolites such as thaxtomins. Cellulases were produced in the presence of suberin only (Figure 1.15C), whereas cellobiose was not produced in the absence of accessible cellulose. Since inducers of thaxtomin biosynthetic genes are lacking, the biosynthesis of thaxtomins is low, depending only on signals promoting morphogenesis and secondary metabolism. In the presence of both suberin and cellulose (Figure 1.15B), suberin may have a dual function in the virulence of *S. scabies*. It may first stimulate the production of cellobiose, the transcriptional inducer of thaxtomin biosynthetic genes, by stimulating cellulase activity and the consequently

the release of cellobiose from cellulose. It may then inhibit the effects of cellobiose on secondary metabolism by acting as a signal molecule for morphogenesis, thereby promoting the production of secondary metabolites such as thaxtomins (Padilla-Reynaud *et al*., 2015).

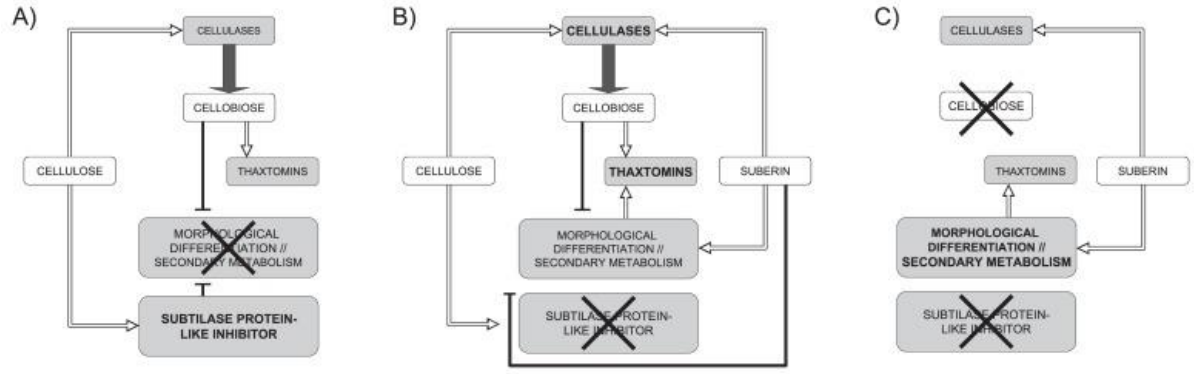


Figure 1.15. Hypothetical mechanism of action of suberin and cellulose on the production of thaxtomin in *S. scabies* (Padilla-Reynaud *et al*., 2015).

1.6.2.2.3.2. Concanamycins

S. scabies produces phytotoxins other than thaxtomins, namely, concanamycins A (Figure 1.16) and B (Natsume *et al*., 1996). Analyses of four taxonomically different *Streptomyces* species that induce common scab of potato showed that only *S. scabies* produces concanamycins, while other species produce only thaxtomins (Natsume *et al*., 1996). Concanamycins are macrolide secondary metabolites that are produced by *S. scabies* and other *Streptomyces* spp. and have been shown to display phytotoxic activity (Haydock *et al*., 2005; Natsume *et al*., 1996). A recent study showed necrosis-inducing activity of concanamycin A and its synergistic action with thaxtomin A in a potato tuber slice assay (Natsume *et al*., 2017). Concanamycins A and B were also detected in the tubers inoculated with *S. scabies* in pot experiments and in field-grown diseased potatoes (Natsume *et al*., 2017). The gene cluster responsible for

concanamycin biosynthesis has already been identified (Haydock *et al*., 2005). The concanamycins exhibit a wide range of important biological activities: antiviral, antiprotozoal and antineoplastic. These activities are thought to be due to their potent inhibition of the vacuolar (V-type) H^+-ATPase (Muroi *et al*., 1993). However, the role of concanamycins in the plant-pathogen interaction is still not fully understood.

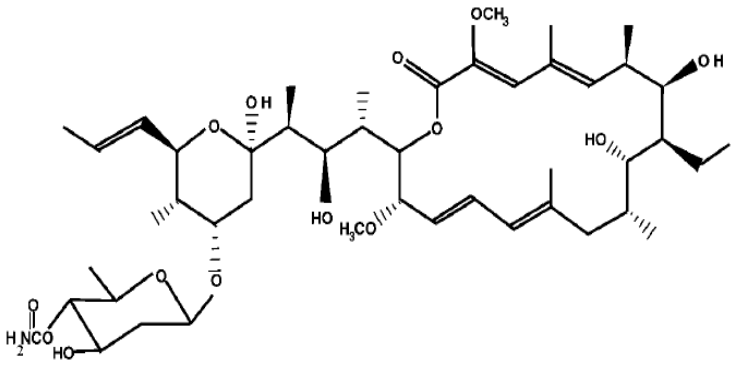


Figure 1.16. Concanamycin A structure (Haydock *et al*., 2005).

1.6.2.2.3.3. Coronafacoyl phytotoxins

The biosynthetic gene cluster predicted to produce coronafacoyl phytotoxins was identified in the genome sequence of *S. scabies* 87.22. Coronafacoyl phytotoxins are non-host-specific phytotoxins that are produced by different plant-pathogenic bacteria (Bignell *et al*., 2018). These metabolites are composed of the bicyclic hydrindane ring-based polyketide coronafacic acid and an amino acid or amino acid derivative linked via an amide bond. Coronatine is the predominant family member of coronafacoyl phytotoxins and the most toxic form (Bignell *et al*., 2018). Coronatine exhibits a wide range of biological activities in plant tissues such as induction of tissue hypertrophy, stimulation of ethylene production, anthocyanin accumulation and inhibition of root elongation. The role of coronafacoyl phytotoxin production in common scab disease development remains unclear. *S. scabies* is the only plant-pathogenic *Streptomyces*

sp. that is able to produce this family of phytotoxins. Although coronafacoyl phytotoxin are not essential for the pathogenicity of *S. scabies* however, it is believed that additional roles for these metabolites remain to be discovered (Bignell *et al*., 2018).

1.6.2.2.4. Degradation of periderm constituents by *S. scabies*

Glycoside hydrolases (also called glycosidases or glycosyl hydrolases) catalyze the hydrolysis of glycosidic bonds in complex sugars present in potato periderm such as cellulose. Glycoside hydrolases can be classified in several ways and the simplest way is based on their substrate specificities. Together with glycosyltransferases, glycosidases form the major catalytic machinery for the synthesis and breakage of glycosidic bonds. The classification based on substrate specificity could be problematic for enzymes that act on multiple substrates. This is particularly true for glycoside hydrolases, which often act on very complex polysaccharides and have a broad spectrum of substrates. For example, endoglucanases, typically considered as cellulases but may also be active on other substrates such as xylan, β-glucan and chitosan, etc. to varying degrees (Henrissat and Davies, 1997). Glycosyl hydrolases may be also classified based on their mode of action on a polysaccharide or based on sequence comparison since there is a direct relationship between sequence similarity and three-dimensional folding and therefore the structure of the protein. The hydrolysis of the β-1,4 glycosidic linkages of the cellulose is carried out by mixtures of hydrolytic enzymes collectively called cellulases. These cellulases include enzymes that synergistically hydrolyze internal linkages (endoglucanases) and external linkages (cellobiohydrolases) (Dashtban *et al*., 2009). The products resulting from these hydrolyses are cellobiose and cellodextrin which inhibit endoglucanase and cellobiohydrolase activity. Thus, the effective hydrolysis of cellulose requires the presence of β-glucosidases to break down the final glycosidic linkages and produce glucose monomers (Maki *et al*., 2009).

In addition to cellulases, *S. scabies* produces many other enzymes that degrade cell wall components such as mannosidases, xylanases/cellulases and cellobioses hydrolases (Joshi *et al*., 2010). Komeil *et al*. (2014) also demonstrated that suberin-enriched potato periderm induced the production of a large amount of glycosyl hydrolases such as cellulases, xylanases and licheninases. This result was unexpected because the substrate contained very few contaminating polysaccharides that act as inducers for the glycosyl hydrolases.

Glycosyl hydrolases, and especially cellulases of *S. scabies*, are of great interest because they may be possibly involved in the pathogenesis. Glycosyl hydrolases would be able to hydrolyze the β bonds of the polysaccharides present in the plant walls. The suberin which was purified from polysaccharides was found to act as an inducer for cellulases better than cellulose itself in *S. scabies* (Komeil *et al*., 2013). Another study by Padilla-Reynaud *et al*. (2015) demonstrated that suberin promoted glycosyl hydrolase activity when added to cellulose-, xylan-, or lichenin-containing media. Also this study showed that the addition of suberin to a cellulose-containing medium increased the production of glycosyl hydrolases (Padilla-Reynaud *et al*., 2015).

1.6.3. Other common scab-inducing species

Potato common scab may be also caused by other species of *Streptomyces*, aside from *S. scabies*, such as *S. acidiscabies* (Lambert and Loria, 1989a), *S. turgidiscabies* (Miyajima *et al*., 1998), *S. stelliscabiei* and *S. europaeiscabiei* (Pasco *et al*., 2005). Both *S. acidiscabies* and *S. turgidiscabies* produce symptoms like those of *S. scabies*, however, they have a more limited geographical distribution than that of *S. scabies*. *S. acidiscabies* has occasionally been reported in the United States and Canada in low pH soils (Faucher *et al*., 1992; Lambert and Loria, 1989a). *S. acidiscabies* causes a scab disease of potato in soils with pH values below 5.2. *S. acidiscabies* is distinct from *S. scabies* in its production of a red or yellow, pH-sensitive diffusible pigment, in the place of melanin. It also grows on agar media at pH 4.0 (versus pH 5.0 for *S. scabies*) and does not use raffinose as a carbon source (Lambert and Loria, 1989a).

S. turgidiscabies was isolated from potato grown in eastern Hokkaido, Japan. It is distinct from other scab-inducing species by having flexuous spore chains and grey mass color. It does not grow on agar media at pH 4.0 or 37 °C and does not produce melanin or other diffusible pigments. It can utilize raffinose and inulin as a carbon source (Miyajima *et al*., 1998). Both *S. scabies* and *S. turgidiscabies* can be found in the same potato cultivars, fields, tubers and also in the same scab lesion (Lehtonen *et al*., 2004).

A large pathogenicity island has been identified and partially sequenced in the genome of *S. turgidiscabies* Car8 (Kers *et al*., 2005). This island was used to identify virulence regions conserved in the *S. scabies* 87.22 genome. The genes associated with the pathogenicity island of *S. turgidiscabies* are found in two remote regions of the genome of *S. scabies* 87.22 (Lerat *et al*., 2009). These two regions are: 1)

toxicogenic region (estimated coordinates *c*. 3596–3653 kb; G+C content of 68%). All genes shown to be involved in thaxtomin biosynthesis are found in this region. These genes are *txtAB* (Healy *et al*., 2000), *txtC* (Healy *et al*., 2000), *nos* (Kers *et al*., 2004) and *txtR* (Joshi *et al*. 2007b). 2) The second segment of the pathogenicity island (PAI), which is called the colonization region contains more genes (estimated coordinates *c*. 8471–8581 kb; G+C content of 68.5%). This chromosomal region contains genes such as *nec1* and *tomA* which are not essential to pathogenicity but play a very important role in virulence. It is believed that these two genes could play a role in the infection process by suppressing the plant defense mechanism (Joshi *et al*., 2007a; Seipke and Loria, 2008). In *S. acidiscabies*, the virulence genes found on the pathogenicity island are localized in two separate regions of the chromosome, called the toxicogenic region and the colonization region like *S. scabies*. The *S. scabies* toxicogenic region (TR) can be further divided into two sub-regions (TR1 and TR2) that are flanked by two attachment (*att*) sites and are separated by an internal *att* site. TR1, which is 20 kb in size, contains the entire thaxtomin gene cluster, and TR2, which is 157 kb, includes putative integrative and conjugative elements (Chapleau *et al*., 2016). It is believed that TR2 is required for mobilization, while TR1 is required for pathogenicity, and both are required for the emergence of new plant-pathogenic species (Zhang *et al*., 2016). The genetic organization of the pathogenicity islands from three common scab-inducing species is illustrated in Figure 1.17.

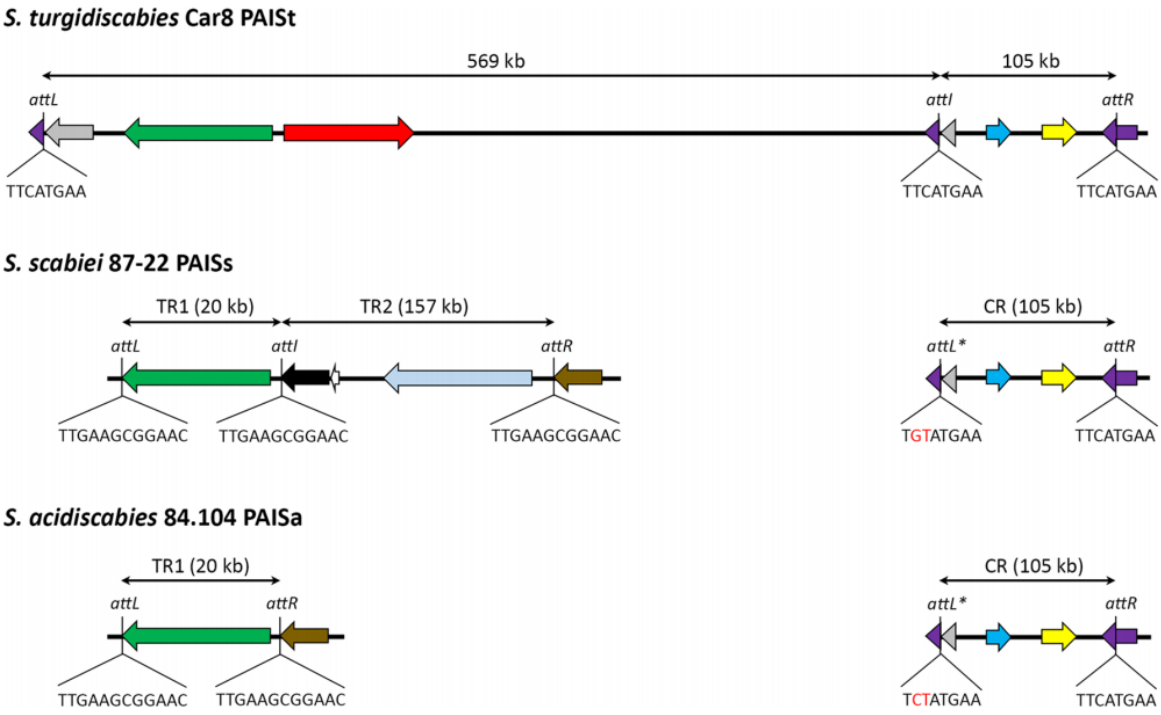


Figure 1.17. Schematic diagram showing the genetic organization of the pathogenicity islands from *Streptomyces turgidiscabies* Car8, *Streptomyces scabies* 87.22 and *Streptomyces acidiscabies* 84.104 (Li *et al.*, 2019).

1.7. Degradation of suberin

Suberin degradation is a complex process which is not fully understood and broadly attributed to fungi. Suberin is known to be recalcitrant to microbial degradation (Kontkanen *et al.*, 2009). This property explains why cork, essentially made of suberin, is the preferred material for wine bottle stoppers.

1.7.1. Degradation of the aliphatic moiety of suberin

Several studies were conducted to characterize the enzymes produced by fungi which are involved in suberin degradation. Degradation of suberin has been shown in some plant pathogenic fungi (Hynes *et al*., 2006) and saprophytic fungi. For example, when *Aspergillus nidulans* was grown in a medium supplemented with suberin, it was shown to utilize suberin as carbon source (Martins *et al*., 2014). The suberin-grown fungus *Fusarium solani* f. sp *pisi* was also found able to generate a cutinase-like esterase which depolymerizes the aliphatic components of suberin (Fernando *et al*., 1984). Raspberry suberin was shown also to be degraded by the two fungal genera *Fusarium solani* f. sp. *pisi* and *Armillaria mellea* (Zimmermann and Eeemüller, 1984). *Coprinopsis cinerea* secreted a cutinase (CcCUT1) that is able to hydrolyze suberin (Kontkanen *et al*., 2009) (Figure 1.18).

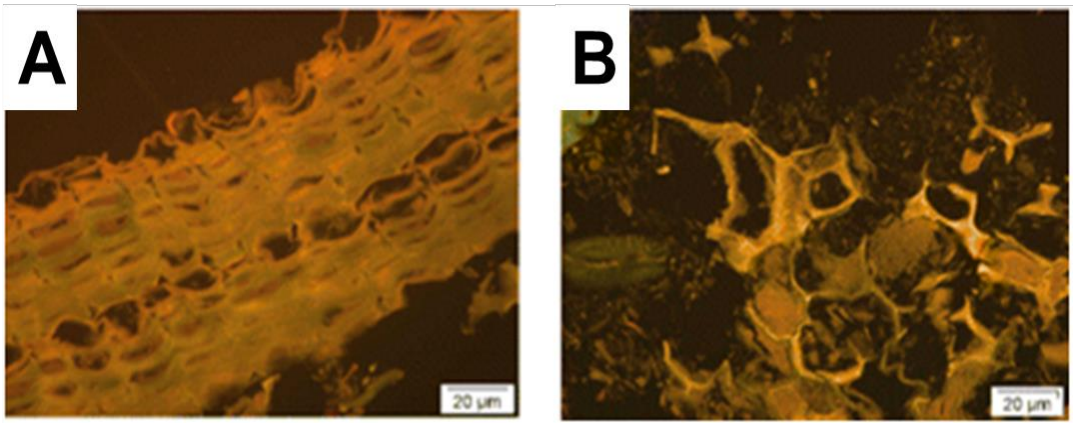


Figure 1.18. Photos of suberin after treatment with cutinase CcCUT1 obtained by fluorescence microscopy. (A) the suberin reference, and (B) suberin treated with CcCUT1 (Kontkanen *et al*., 2009).

A study by Ofong and Pearce (1994) showed the release of extractable suberin monomers from plant tissues (*Salix repens* rhytidome or purified potato periderms) following prolonged incubation (5 months) with the fungus *Rosellinia desmazieresii*. In their study, long-chain aliphatic monomers were detected using gas liquid chromatography, in culture filtrates of *R. desmazieresii* grown on *S. repens* rhytidome. Very low concentrations of C_{14}, C_{16}, C_{18} and C_{20} compounds were present, and traces of longer chain molecules were detectable which were absent in filtrates from uninoculated flasks. These compounds were present in the chemical depolymerisation products from suberin. These results confirm the degradation of suberin. Similarly, when cultures of *Fusarium solani* f. sp. *pisi* and *Armillaria mellea* were grown in a medium supplemented with 0.5 % suberin isolated from raspberry periderm, the culture supernatant of both fungi were found to contain suberin monomers like fatty alcohols and acids with chain-lengths from C_{16} to C_{26}, as well as C_{16} and C_{18} ω-hydroxyacids which could be considered as suberin degradation products (Zimmermann and Eeemüller, 1984).

The degradation of suberin could be explained by the ability of these fungi to synthesize esterases which are able to degrade the aliphatic part of suberin. Their presence in the culture medium was detected by measuring esterase activity. The fungus *Rosellinia desmazieresii*, causing of a ring-dying disease of *Salix repens* was found to secrete esterases in culture medium supplemented with *S. repens* rhytidome (2.0 g) and potato suberin (0.5 g) (Ofong and Pearce, 1994). The extracellular esterases from culture filtrates and cell-bound esterases from mycelial washings were assayed using *p*-nitrophenyl butyrate as a chromogenic substrate. Cell-wall-degrading enzymes, especially esterases capable of degrading suberised phellem tissues, are likely to be important for infection in those fungi that can directly penetrate and colonize the bark of woody plants. *R. desmazieresii* is one of the relatively few fungi that are known to infect in this manner (Ofong and Pearce, 1994). Martins *et al*. (2014)

reported that during the fungal growth of saprophytic fungus *Aspergillus nidulans* on suberin, cutinase 1 and some lipases (e.g. AN8046) acted as the major suberin degrading enzymes. Esterases that degrade suberin belong to cutinases and some cutinases were shown to degrade both suberin and cutin such as CcCUT1 which is a cutinase produced by the fungus *Coprinopsis cinerea* and is able to degrade suberin (Kontkanen *et al*., 2009).

The plant pathogenic bacterium, *S. scabies* was shown to be involved in the degradation of aliphatic part of suberin (Beaulieu *et al*., 2016). This study showed also physical, chemical and proteomic evidence of degradation of suberin by *S. scabies* strain EF-35 and suberin degradation was observed under electron microscopy (Beaulieu *et al*., 2016) (Figure 1.19).

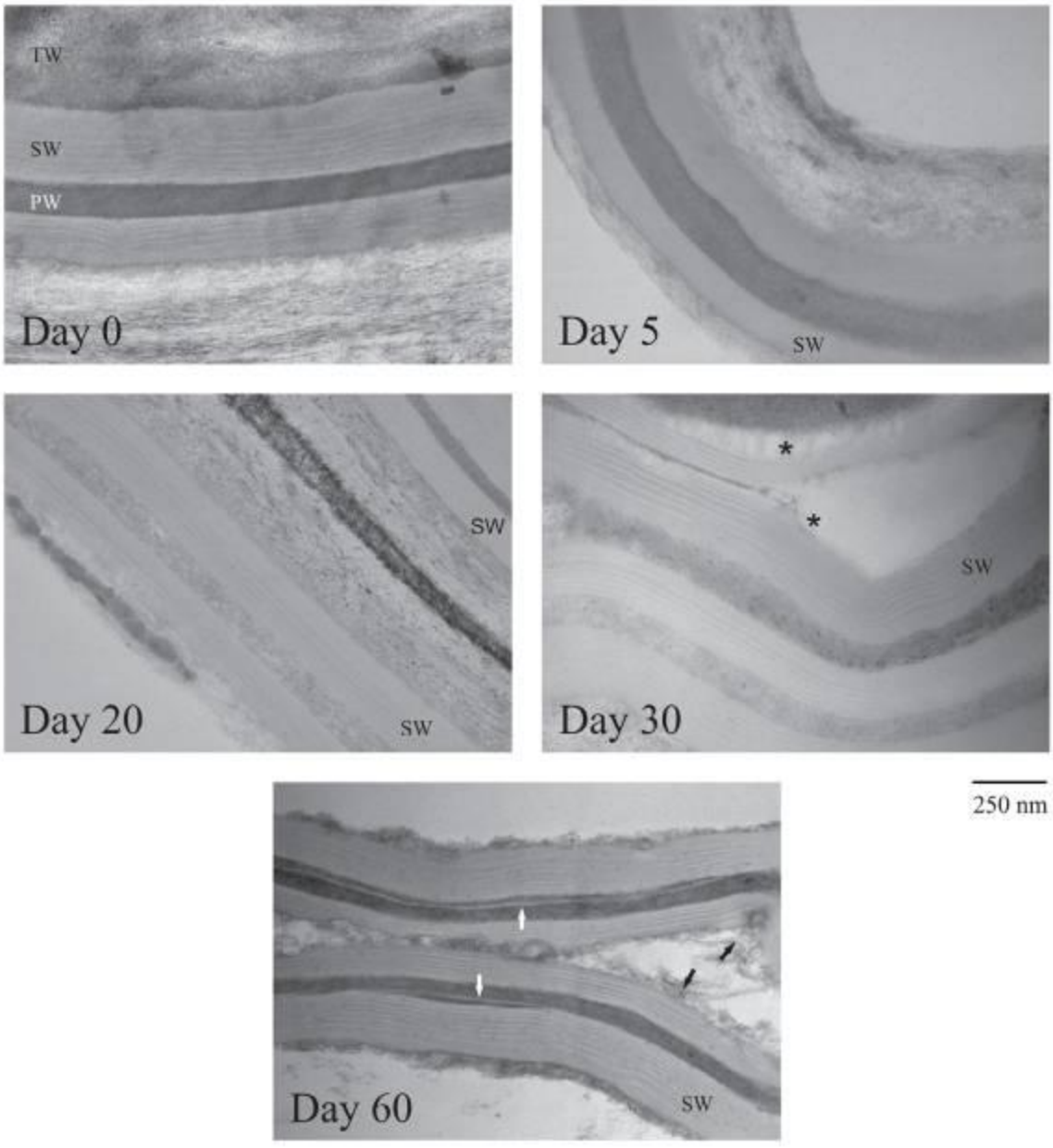


Figure 1.19. Electron micrographs of suberin-enriched potato periderm incubated with *S. scabies* EF-35 for 0 to 60 d. PW: primary cell wall; SW: suberized secondary cell wall; TW: tertiary cell wall. * shows the zone of secondary wall detachment. White arrows show suberin lamellae detached from the secondary wall. Black arrows show broken suberin lamellae (Beaulieu *et al*., 2016).

S. scabies strain EF-35 showed a high esterase activity when grown in the presence of suberin (Figure 1.20) (Komeil *et al*., 2013). In addition, the genomic analysis of this strain revealed the presence of a gene named *sub1* with a significant similarity with the gene coding for cutinase CcCUT1 of *Coprinopsis cinerea* VTT D-041011, a protein for which a suberinase activity has been demonstrated (Komeil *et al*., 2013; Kontkanen *et al*., 2009). The expression of this gene (*sub1*) was found to be specifically induced in the presence of both suberin and cutin (Komeil *et al*., 2013). This specificity was demonstrated following gene expression study of the *sub1* gene of *S. scabies* EF-35 grown in the presence of various carbon sources (suberin, cutin, starch). Furthermore, analysis of the secretome of *S. scabies* in the presence of suberin as carbon source has identified several proteins that could be involved in the degradation of the aliphatic portion. These are two esterases (C9ZG71 and C9Z6Y6) and an esterase-lipase (C9YTK3) (Komeil *et al*., 2014). Jabloune (2018) purified and characterized the protein encoded by the *sub1* gene. This protein was identified as a suberinase which was able to release fatty acids from both cutin and suberin under the action of the purified sub1 enzyme. Sub1 represents the first bacterial enzyme for which activity on suberin was demonstrated (Jabloune, 2018).

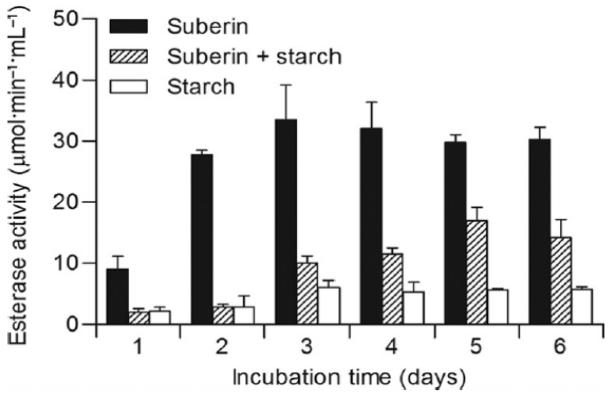


Figure 1.20. Esterase activity of culture supernatants of *S. scabies* EF-35 grown in minimal medium supplemented with 0.5% suberin, 0.5% suberin + 0.5% starch, and 0.5% starch (Komeil *et al*., 2013).

1.7.2. Degradation of the aromatic moiety of suberin

It has been suggested that the release of ferulic acid during fungal growth on potato suberin involve the activity of feruloyl esterases (Fae) (Kolattukudy, 2001). When the saprophytic fungus *Aspergillus nidulans* was grown in minimal media containing 0.1% w/v of suberin as sole carbon source, up-regulation of feruloyl esterase C (*faeC*) (AN5267) was monitored as a result of probable release of ferulic acid (Martins *et al*., 2014). Moreover, other genes encoding proteins that are believed to be involved in ferulic acid degradation were upregulated in the presence of suberin such as the gene *dhbD* (AN6723) which is encoding for 2,3-dihydroxybenzoate carboxylyase (Martins *et al*., 2014). Several downstream products of phenolic suberin degradation have been reported in different fungal strains, however the associated enzymes remain largely unknown (Mathew and Abraham, 2006). Martins *et al*. (2014) has reported the ability of the fungus *Aspergillus nidulans* to degrade the aromatic part of suberin by monitoring the degradation products when it was grown on suberized cell walls.

To our knowledge, there is no report in the scientific literature that a bacterium can degrade the aromatic part of suberin. However, Our research group previously reported that the secretome analysis of the bacterium *S. scabies* during growth in the presence of potato suberin revealed the presence of two feruloyl esterases (C9ZE96 and C9YVP7) that could be involved in the disjunction of the aliphatic and aromatic domains of suberin (Komeil *et al*., 2014) and also one protein (C9Z2P6) involved in the degradation of aromatic compounds was detected in the presence of suberin (Komeil *et al*., 2014).

Despite the fact that no streptomycetes were shown to be able to degrade the aromatic part of suberin, several species of *Streptomyces* are known to degrade aromatic

compounds. *Streptomyces* sp. strain ERI-CPDA-1 (isolated from oil contaminated soil) is able to degrade polycyclic aromatic hydrocarbons (PAHs) (Balachandran *et al*., 2012). *Streptomyces setonii* strain ATCC 39116 was shown to degrade the single aromatic compounds (Park and Kim, 2003). Hydroxycinnamic acids, which are the main constituents of suberin aromatic domain (Bernards *et al*., 1995), were also shown to be degraded by several *Streptomyces* species such as *Streptomyces sannanensis* (Ghosh *et al*., 2007), *Streptomyces viridosporus* (Davis and Sello, 2010) and *Streptomyces setonii* (Sutherland *et al*., 1983).

1.8. Objectives of this work

The first goal of this project was to produce and purify the Sub1 protein in addition to the characterization of its enzymatic properties. Moreover, the study was to be extended to demonstrate that Sub1 acts as a polyesterase able to depolymerize the aliphatic part of suberin and cutin, as well as the synthetic polyester PET.

As the ability of this pathogen to degrade the aromatic part of suberin is still unknown, the second goal of this project was to test the ability of *S. scabies* to degrade the *trans*-ferulic and *p*-coumaric acids; the main constituents of the suberin aromatic domain. A hypothetic degradation pathway of these two compounds was then to be proposed based on a proteomic study, coupled with gene expression analysis.

The third goal of this project was to investigate the interaction between three common scab-inducing species and suberin-enriched potato periderm. *S. scabies* 87.22 and the emerging common scab-inducing species (*S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8) were to be compared for both their ability to grow on suberin-enriched potato periderm and to retrieve nutrients from this substrate.

CHAPTER 2

ENZYMATIC DEGRADATION OF *P*-NITOPHENYL ESTERS, POLYETHYLENE TEREPHTHALATE, CUTIN, AND SUBERIN BY SUB1, A SUBERINASE ENCODED BY THE PLANT PATHOGEN *STREPTOMYCES SCABIES*

2.1. Preface

Previous studies have suggested that the plant pathogen *S. scabies* is able to degrade the plant polymer suberin. This bacterium exhibited a strong esterase activity in the presence of suberized tissues suggesting its ability to degrade the aliphatic part of suberin. The sub1 protein of *S. scabies* showed a similarity with the cutinase CcCUT1 of the fungus *Coprinopsis cinerea*. Moreover, sub1 gene expression has been reported to be specifically induced in the presence of suberin.

The results of this work established that the sub1 gene of *S. scabies* encodes a protein acting as a suberinase which was able to degrade the suberin aliphatic moiety. The sub1 protein was able to release fatty acids from both suberin and cutin. The identified enzyme could have various industrial applications since it was also able to hydrolyze various synthetic substrates.

The results of this work are presented in Section 2.2 and submitted to the journal Microbes and Environments, under the title "Enzymatic degradation of *p*-nitrophenyl esters, polyethylene terephthalate, cutin, and suberin by Sub1, a suberinase encoded by the plant pathogen *Streptomyces scabies*". The authors of the manuscript are:

Raoudha Jabloune, Mario Khalil, Issam E. Ben Moussa, Anne-Marie Simao-Beaunoir, Sylvain Lerat, Ryszard Brzezinski and Carole Beaulieu.

MK contributed to this paper by participation in purification of Sub1 enzyme and performing the enzymatic assays on suberin, cutin and polyethylene terephthalate (PET).

2.2. Manuscript of the article

Title: Enzymatic degradation of *p*-nitrophenyl esters, polyethylene terephthalate, cutin, and suberin by Sub1, a suberinase encoded by the plant pathogen *Streptomyces scabies*.

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Abstract

The genome of *Streptomyces scabies*, the predominant causal agent of potato common scab, possesses a potential cutinase, protein Sub1, whose expression was previously shown to be specifically induced in the presence of suberin. The *sub1* gene was expressed in *Escherichia coli* and the recombinant protein Sub1 was purified and characterized. The enzyme was shown to be versatile as it can hydrolyze various natural and synthetic substrates. Sub1 was able to hydrolyze *p*-nitrophenyl esters, with the hydrolysis of those harboring short carbon chains being the most effective. The V_{max} and K_m values of Sub1 for *p*-nitrophenyl butyrate were 2.36 mol/g/min and 5.7 10^{-4} M, respectively. Sub1 was shown to hydrolyze the recalcitrant polymers cutin and suberin as the release of fatty acids from these substrates was measured following the incubation of the enzyme with these polymers. Furthermore, the hydrolyzing activity of the esterase Sub1 on the synthetic polymer polyethylene terephthalate (PET) was demonstrated by the release of terephthalic acid (TA). Sub1 activity on PET was substantially enhanced in the addition of Triton and was shown to be stable for at least 20 days at 37°C.

Keywords: actinobacteria, common scab, cutinase, esterase, potato

Introduction

Streptomycetes are Gram-positive bacteria known for their ability to produce a wide range of secondary metabolites and for the complexity of their morphological development. Although most streptomycetes species are saprophytic soil inhabitants, some are plant pathogens. Among them, *Streptomyces scabies* is the predominant causal agent of potato common scab and causes important economic losses in Canada (18), as well as in most potato growing areas. Common scab is characterized by corky lesions on the surface of potato tubers. Like other soil-inhabiting streptomycetes, *S. scabies* produces a large variety of extracellular enzymes including various glycosyl hydrolases and esterases (2, 23). These enzymes may participate in pathogenesis, as penetration of *S. scabies* into host plants is thought to be facilitated by the secretion of extracellular cell wall-degrading enzymes (3).

The potato tuber is covered by a periderm composed of three types of tissues: phellem, phellogen and phelloderm (41). The cell wall of phellem cells is impregnated with suberin, a plant polymer recalcitrant to bio-degradation, composed of a polyaromatic domain covalently linked to a polyaliphatic moiety (5). The polyaromatic domain, a lignin-like structure, consists of a hydroxycinnamic acid-derived polymeric matrix (6). The polyaliphatic domain shares structural and chemical similarities with cutin, another polyester component of plant cuticles. Both cutin and suberin act as physical barriers against plant pathogens (19). Cutin and suberin are polymers of fatty acid derivatives linked by ester bonds. Cutin is mostly composed of C16 and C18 ω-hydroxyacids, polyhydroxyacids, epoxyacids and α,ω-dicarboxylic acids. Suberin can be distinguished from cutin by higher contents of hydroxycinnamic acids, fatty alcohols and saturated aliphatics with long chains (4).

Cutinases hydrolyze the plant leaf cuticle by cleaving the ester bounds of cutin (13). As such, cutinases belong to the esterase group. More specifically, cutinases belong

to a class of serine esterases that contain the catalytic triad (serine, histidine and aspartate) with the active serine in the consensus sequence Gly-His/Tyr-Ser-X-Gly (30). Some fungal cutinases such as cutinase CcCUT1 of the fungus *Coprinopsis cinerea*, also show the ability to degrade suberin (24). As lipolytic enzymes, cutinases present interesting properties for applications in various industrial processes (8). For instance, some cutinases exhibit the ability to degrade synthetic polyesters such as polycaprolactone (33) and polyethylene terephthalate (PET) (14, 36, 43).

Some studies have suggested that the bacterium *S. scabies* possesses the ability to degrade suberin. This pathogenic bacterium exhibits strong esterase activity in the presence of suberized tissues (3). Furthermore, the secretome analysis of *S. scabies* cultures grown in the presence of suberin revealed the presence of esterases predicted to play a role in lipid metabolism (2, 22). Among them, the Sub1 protein exhibits 33% identity with cutinase CcCUT1 of *C. cinerea* (23). Interestingly, *sub1* gene expression has been reported to be specifically induced in the presence of suberin (23). The cutinase from *Aspergillus oryzae* (PDB ID: 3GBS) is hallmarked by a central β-sheet of five parallel strands surrounded by ten α-helices, as found for the predicted three-dimensional structure of the Sub1 protein (Fig. 2.1). The model of the Sub1 protein also predicts the formation of two disulfide bonds (Cys31–Cys103; Cys178–Cys185) and a catalytic triad including residues Ser 114, Asp 182 and His 195 (23).

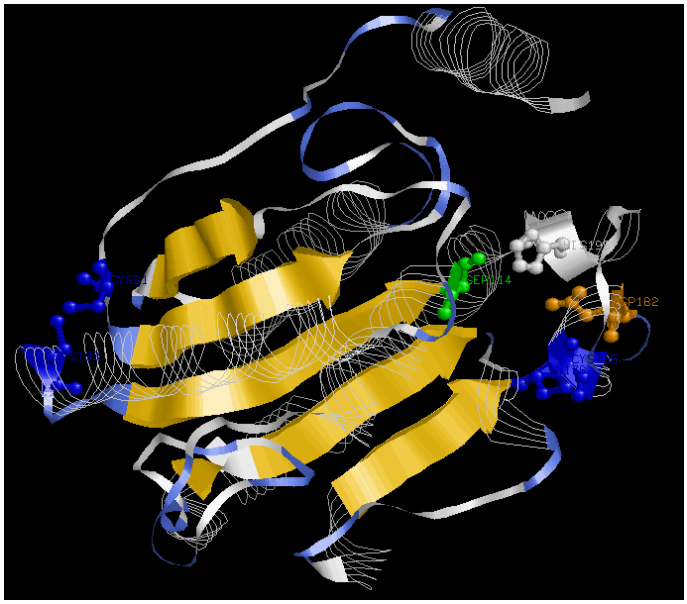

Figure 2.1. Three-dimensional structural model of the Sub1 protein. The structural model was obtained submitting the deduced amino acid sequence of Sub1 (devoided of the signal peptide segment) to the ESyPred3D server (26). The graphic was executed with the RasMol program (38). Color legend: yellow, β-sheets; white, α-helices; blue, disulfide bonds; and green-brown-white, catalytic triad.

The main objectives of this work were to produce the Sub1 protein, to purify and characterize its enzymatic properties and to demonstrate that Sub1 acts as a polyesterase able to degrade biopolymers such as cutin and suberin, as well as the synthetic polyester PET.

Materials and Methods

Bacterial strains and culture conditions

Inoculum of *Streptomyces scabies* EF-35 (HER1481) was prepared in tryptic soy broth (10^8 spores in 25 mL), as described by Komeil et al. (23). Cultures of *S. scabies* EF-35 were incubated with shaking (250 rpm) at 30°C. *Escherichia coli* strains DH5α (Invitrogen) and SHuffle T7 (New England Biolabs) were grown in LB medium supplemented when necessary with kanamycin (30 µg/mL) and were incubated with shaking (250 rpm) at 37°C.

Suberin and cutin preparation

Suberin-enriched potato periderm was obtained as previously described (20). The extracted material was dried under a hood, ground using a coffee mill and stored at room temperature. To further remove residual polysaccharides in potato periderm, this material was exposed to microbial degradation in the presence of *S. scabies* EF-35, as described by Beaulieu et al. (2). *Streptomyces scabies* inoculum (1 mL) was added to 50 mL of minimal medium consisting of a mineral solution (0.5 g/L $(NH_4)_2SO_4$; 0.5 g/L K_2HPO_4; 0.2 g/L $MgSO_4$-$7H_2O$ and 10 mg/L $FeSO_4$-$7H_2O$) and 1 g/L of suberin-enriched potato periderm. After 30 days of incubation, both 10 mL of fresh mineral solution and 200 µL of *S. scabies* inoculum were added to the culture and the incubation was extended for an additional 30 days. The culture was centrifuged at 3450 *g* for 20 min and the pellet was resuspended in 100 mL of sterile water, and autoclaved for 15 min. The suspension was washed with sterile water to remove bacterial cells debris. The resulting material (purified potato suberin) was dried at 50°C

for 24 h. Cutin was isolated from apples following the protocol of Walton and Kolattukudy (44).

DNA extraction

Genomic DNA was isolated from 48-h bacterial cultures of *S. scabies* EF-35 using the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich) according to the manufacturer's instructions. Plasmid DNA was isolated from 12-h *E. coli* cultures using GenElute™ Plasmid Miniprep Kit (Sigma-Aldrich) following the manufacturer's instructions.

Cloning of sub1 in *Escherichia coli*

The *sub1* coding sequence, deprived of its signal peptide (GenBank accession number MK689853), was amplified by PCR from genomic DNA of *S. scabies* EF-35 using primers F-pET (5'-ATATCCATGGCCGCCTGCACGGACATCG-3') and R-pET (5'-ATATCTCGAGTTAGATCTTGGTCGCGGCGAAGG-3'). The PCR mix contained 20 ng of DNA, 2.5 µL of *Taq* polymerase buffer, 0.5 µL of dNTPs (10 mM), 0.5 µL (each) of forward and reverse primers (10 µM), and 0.125 µL of DNA *Taq* polymerase (New England Biolabs), in a total volume of 25 µL. PCR conditions consisted of 2 min at 95°C followed by 30 cycles at 95°C for 30 s, 1 min at 64°C, 1 min at 68°C, with a final extension of 5 min at 68°C. PCR was performed using the thermocycler T100 (Bio-Rad). Amplification products were migrated on 1% agarose gel (37), purified from gels using MinElute Gel Extraction Kit (Qiagen) and cloned into the pET-30-a(+) vector (Novagen). The amplication product and the cloning vector pET-30a(+) were both digested using restriction enzymes *Nco*I and *Xho*I. The enzyme T4 DNA ligase (New England Biolabs) was used to ligate plasmids ends to amplicons following the

manufacturer's instructions. Ligation products were heat shock-transformed into competent cells of *E. coli* DH5α as per the manufacturer instructions (New England Biolabs). Bacteria were then incubated overnight on LB agar medium supplemented with kanamycin (30 µg/mL). The plasmid insert was sequenced at sequencing and genome genotyping platform (CHUL, University Laval, Quebec City, Canada). Plasmid pET-30a(+), with or without the *sub1* insert, was transformed into the expression host *E. coli* SHuffle T7, as previously described.

Protein extraction

Cultures of *E. coli* SHuffle T7 carrying pET without or with the *sub1* gene insert (*E. coli* SHuffle T7-pET and *E. coli* SHuffle T7-pET-*sub1*) were incubated on LB agar medium supplemented with kanamycin. When the OD_{600} reached 0.6-0.8, isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to the culture (0 to 1.0 mM, final concentration) and bacteria were incubated at 25°C for an additional 24 h. Cells were harvested by centrifugation (3450 *g*) for 10 min, pellets were washed twice with saline (NaCl 0.9%) and resuspended in a buffer solution (NaH_2PO_4 50 mM and NaCl 300 mM, pH 8.0) supplemented with EDTA (2.5 mM). The suspensions were sonicated on ice four times for 10 s and centrifuged (3450 *g*) for 30 min at 4°C to remove cell debris. The supernatant was collected and successively passed through 0.45 µm and 0.2 µm pore size filters. The resulting protein solution was stored at 4°C.

Purification of protein Sub1

An affinity column Ni-NT cOmplete His-Tag purification column (Roche) was used to purify protein Sub1 from the cytoplasmic fraction of *E. coli* SHuffle T7-pET-*sub1*, following the manufacturer's instructions. Elution buffer A (50 mM NaH_2PO_4, 300 mM NaCl, pH 8.0), supplemented with different concentrations of imidazole (5 to 250 mM), was used for column washing and His6-tagged protein elution. Protein fractions were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) migration along with marker PageRuler™ Prestained Protein Ladder (Thermo Scientific), as described by Komeil et al. (22). Proteins were stained with Coomassie brilliant blue R-250 (Bio-Rad; 27) and fractions containing purified Sub1 were pooled. This mixture was dialyzed in a phosphate buffer solution (PBS) to remove imidazole. Protein concentration was measured according to Bradford (7).

Esterase activity of Sub1 on *p*-nitrophenyl esters

Esterase activity was determined by measuring spectrophotometrically the absorbance of *p*-nitrophenol using substrates *p*-nitrophenyl butyrate (C4), *p*-nitrophenyl octanoate (C8), *p*-nitrophenyl decanoate (C10) and *p*-nitrophenyl dodecanoate (C12) (Sigma-Aldrich). The molar extinction coefficient of *p*-nitrophenol in Tris-HCl (20 mM, pH 7.5) at room temperature is 12000 M^{-1} cm^{-1} at 420 nm. This enzymatic assay was performed as described previously (23) with slight modifications. In a 1.5 mL plastic cuvette, 20 µL of 100×-diluted Sub1 (60 ng) were added to 970 µL of Tris-HCl (20 mM, pH 7.5), containing or not Triton X-100 (0.5%), and 10 µL of a 20 mM *p*-nitrophenyl ester substrate (0.2 mM final concentration). The absorbance at 420 nm of this reaction mix was measured at room temperature every 10 s for 1 min. The increase of absorbance of each sample was read against a blank without purified

protein. One unit (U) was the amount of enzyme liberating 1 µmol of *p*-nitrophenol per min under the assay conditions. V_{max} and K_m values of enzyme Sub1 were determined using software GraphPad Prism7, according to the Michaelis-Menten equation, with different concentrations of the C4 substrate.

Esterase activity of Sub1 on natural and synthetic polymers

Suberin and cutin were exposed to enzyme Sub1 as follows. Suberin or cutin (10 mg) was added to 350 µL of Tris-HCl (20 mM, pH 7.5) supplemented with 50 µL of purified enzyme Sub1 (15 µg). Control assays (blanks) were made of suberin and cutin without the addition of enzyme Sub1**.** The mixture was incubated at room temperature for 20 days. Colorimetric assays of free fatty acids released from the biopolymer were performed every 5 days using Free Fatty Acid Quantification Colorimetric/Fluorometric Kit (BioVision) according to the manufacturer's instructions. A standard curve was prepared with palmitic acid to convert the absorbance at 570 nm into fatty acid concentration.

The hydrolyzing activity of Sub1 was also estimated on polyethylene terephthalate (PET) by measuring the amount of terephthalic acid (TA) released from PET according to Nimchua et al. (34) with slight modifications. Assays were conducted in 2 mL tubes containing 10 mg of PET (ground granules, Sigma-Aldrich), 1 mL of Tris-HCl (20 mM, pH 7.5), 3 µg of enzyme Sub1. In a first experiment, the effect or Triton on Sub1 performance was tested. Tubes, containing or not Triton X-100 (0.5%), were incubated at 37°C and the concentrations of TA released in the incubation media were recorded after 10 and 15 days. Blank assays, in which enzyme Sub1 was omitted, were used as controls. Tubes were then centrifuged (1 min) and 50 µL of the collected supernatant were added to 350 µL of Tris-HCl (20 mM, pH 7.5) into quartz cuvettes.

Absorbance at 240 nm was measured to determine TA concentration (using a standard curve). In another experiment, the stability of Sub1, using PET as substrate, was assessed at 37°C and 50°C over a 20-day period. The incubation medium contained Triton X-100 (0.5%) and the concentration of TA released in the reaction mix was measured every 5 days, as described above.

Results

Heterologous production of Sub1

The *Streptomyces scabies sub1* gene was cloned into a pET expression vector (data not shown) and expressed in *E. coli* strain SHuffle T7. The cytoplasmic extract of *E. coli* SHuffle T7-pET-*sub1* was characterized by a thick and dense band of approximatively 25 kDa which was absent from the protein profile of *E. coli* SHuffle T7-pET (Fig. 2.2). In *E. coli* SHuffle T7-pET-*sub1*, Sub1 was produced even in the absence of IPTG, but thicker protein bands were observed when the *sub1* gene expression was induced by this compound (Fig. 2.2).

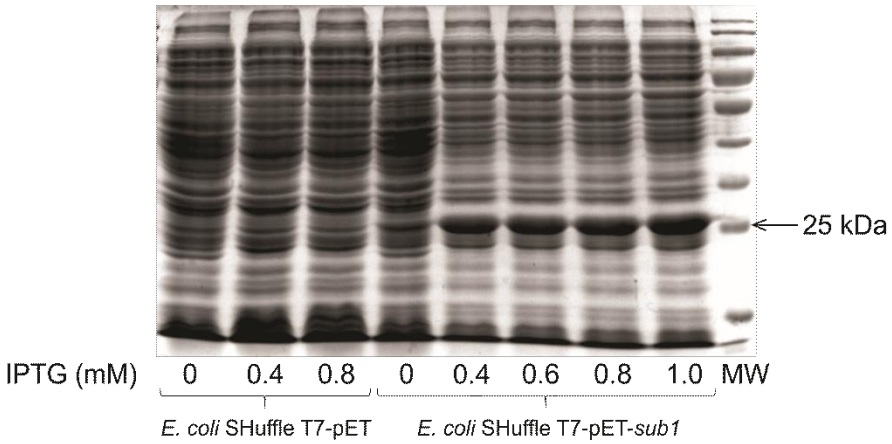

Figure 2.2. SDS-PAGE gel of the cytoplasmic extract obtained from pET-transformed *Escherichia coli* strain SHuffle T7, without (*E. coli* SHuffle T7-pET) or with (*E. coli* SHuffle T7-pET-*sub1*) the insert of the *sub1* gene, after induction with different concentrations of IPTG.

The cytoplasmic fraction from cultures of *E. coli* SHuffleT7-pET-*sub1* showed, in the absence of induction with IPTG, an esterase activity on the C4 substrate (58.8 µmol/mL) significantly higher than in control *E. coli* SHuffle T7-pET (1.6 µmol/mL) after 30 min of incubation (Fig. 2.3). Esterase activity of *E. coli* SHuffle T7-pET-*sub1* was inducible with IPTG and was quickly observable in the reaction mix. After 5 min incubation, the highest activity reached 32.2 µmol/mL when 0.8 mM IPTG was added to the culture, compared to 6.3 µmol/mL in the absence of IPTG (Fig. 2.3).

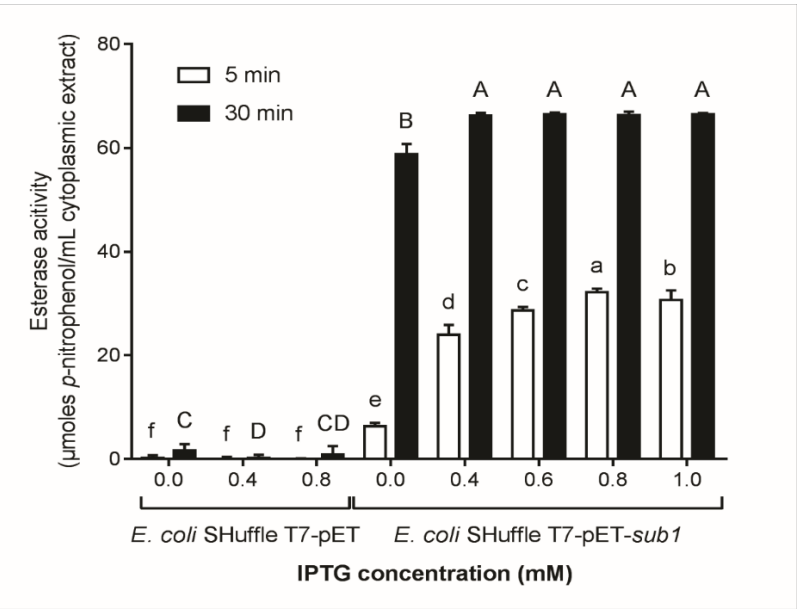

Figure 2.3. Esterase activity of cytoplamic extracts from *Escherichia coli* SHuffle T7 transformed with plasmid pET without (*E. coli* SHuffle T7-pET) or with (*E. coli* SHuffle T7-pET-sub1) the *sub1* insert and exposed to various concentrations of IPTG. The activity is expressed as concentration of *p*-nitrophenol released from *p*-nitrophenyl butyrate substrate in 5 min and 30 min reactions. These results are means of five replicates ± SD. Bar values accompanied by the same lower case letter or upper case letter are not significantly different.

Purification of the recombinant protein His-Sub1

An affinity column Ni-NT (cOmplete His-Tag) was used to purify the recombinant protein Sub1 from the cytoplasmic fraction of *E. coli* SHuffle T7-pET-*sub1*. By comparing the migration profile of the cytoplasmic fraction and the flow-through (Fig. 2.4, lanes 2 and 3, respectively), it was observed that the band corresponding to the recombinant protein His-Sub1 (25 kDa) was present in the cytoplasmic fraction, while no band was present in the flow-through fraction, indicating that the Sub1 protein

bound the column. The presence of imidazole in the elution buffer allows the elution of His-tag proteins from the column. At imidazole concentrations ranging from 4 to 10 mM, the elution buffer released the majority of the contaminant proteins while a low quantity of Sub1 was released with 10 mM imidazole (lanes 4-7, Fig. 2.4). A detectable quantity of Sub1 was eluted with no contaminant proteins when 50 mM imidazole buffer was used (lane 8, Fig. 2.4). However, at 200 mM imidazole, no recombinant protein was detected (lane 9, Fig. 2.4). Mass spectrometry analysis confirmed that the purified protein was Sub1 (data not shown). The purification efficacy was estimated by comparing the esterase activity of the cytoplasmic fraction and the purified protein. The esterase activity of Sub1 in purified extract (23.0 U/mL) was 52-fold higher than the cytoplasmic crude extract (0.4 U/mL).

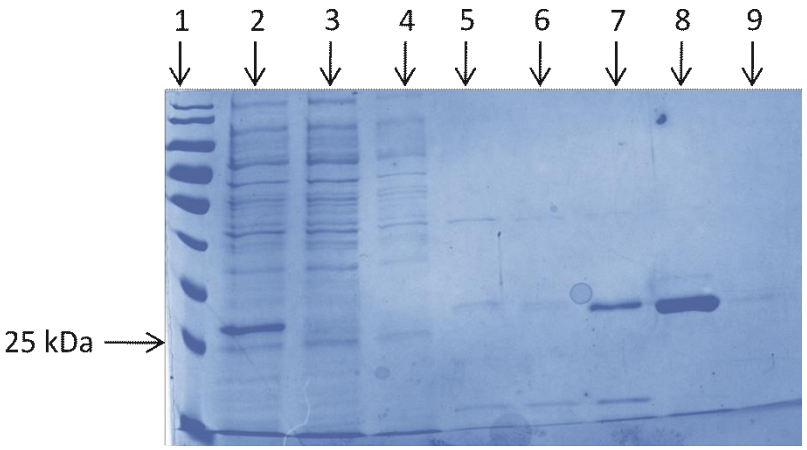

Figure 2.4. SDS–PAGE gel of the cytoplasmic soluble proteins obtained from *Escherichia coli* transformed with SHuffle T7-pET-*sub1*, after fractionation on the affinity column (IMAC). Lane 1, molecular weight marker; lane 2, cytoplasmic extract; lane 3, flow-through; lane 4, proteins released after washing with buffer A; lanes 5 to 9, proteins released after washing with buffer A supplemented with 4, 5, 10, 50 or 200 mM imidazole, respectively.

Esterase activity of the Sub1 protein on *p*-nitrophenyl esters

Esterase activity of Sub1 on *p*-nitrophenyl esters of varying carbon chain lengths was determined in the presence and absence of Triton X-100. Independent of the presence of Triton X-100, Sub1 was more active on *p*-nitrophenyl butyrate (C4) and *p*-nitrophenyl octanoate (C8) than on *p*-nitrophenyl esters with longer carbon chains (C10 and C12, Fig. 2.5). The presence of Triton X-100 increased esterase activity of the enzyme on all of these substrates (Fig. 2.5).

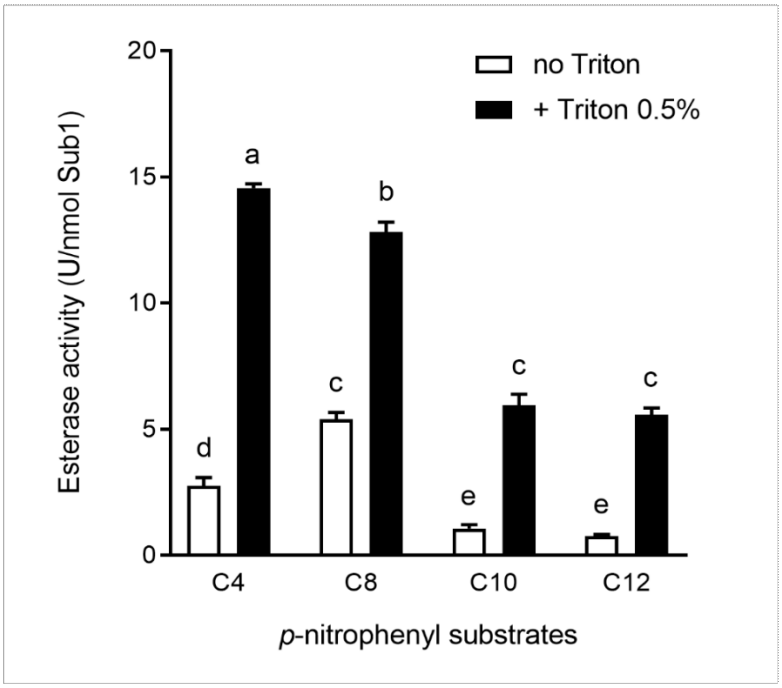

Figure 2.5. Esterase activity of the purified Sub1 enzyme using *p*-nitrophenyl substrates of different carbon chain sizes (C4, C8, C10 and C12) in the absence or presence of Triton X-100 (0.5%). Data shown are the mean ± SD of three replicates. Bar values accompanied by the same letter are not significantly different.

The highest esterase activity (14.6 U/nmol or 616 U/mg Sub1), was obtained on the C4 substrate in the presence of Triton X-100. Thus, V_{max} and K_m values for esterase Sub1 were calculated using the latter substrate. As shown in Fig. 2.6, the initial velocity (V_0) of the hydrolysis reaction increased when higher concentrations of the substrate *p*-nitrophenol butyrate (*p*-NPB) were used. According to the Michaelis-Menten equation, V_{max} was 55.8 ± 2.0 U/nmol Sub1 (2361 ± 84.5 U/mg Sub1) and K_m was 0.57 ± 0.04 mM *p*-NPB.

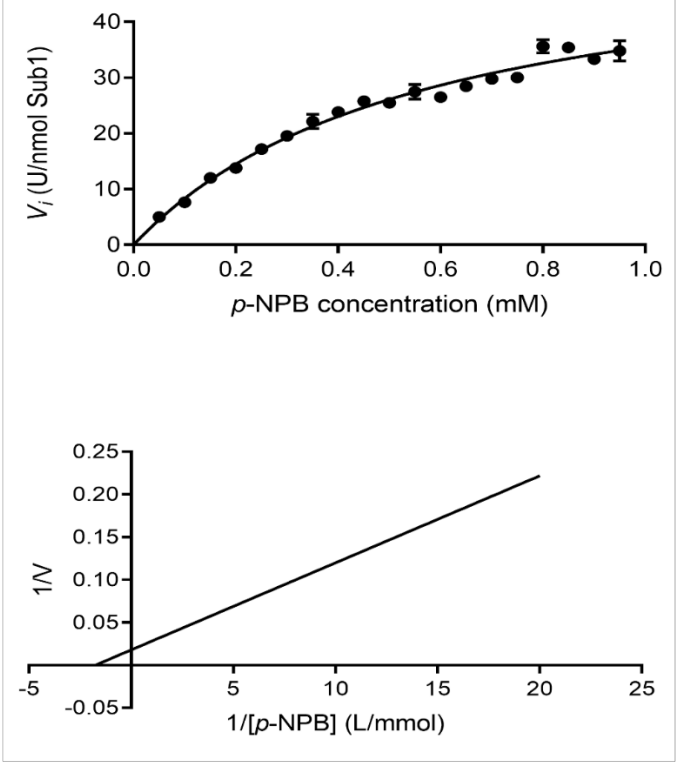

Figure 2.6. Effect of the substrate (*p*-NPB) concentration on the initial speed (V_0) of the hydrolysis reaction of the esterase Sub1. These results are means ± SD of three replicates.

Effect of the Sub1 esterase on polymers

Sub1 was shown to hydrolyze suberin and cutin, releasing 1.22 ± 0.06 nmol and 2.65 ± 0.18 nmol of fatty acids (palmitic acid equivalent) per µg of Sub1 from these polymers, respectively, after 20 days of incubation. Sub1 ability to degrade cutin and suberin appeared stable over the experimental time course, at room temperature, as the amounts of fatty acids released in the incubation medium were correlated with time ($P < 0.0001$; Fig. 2.7).

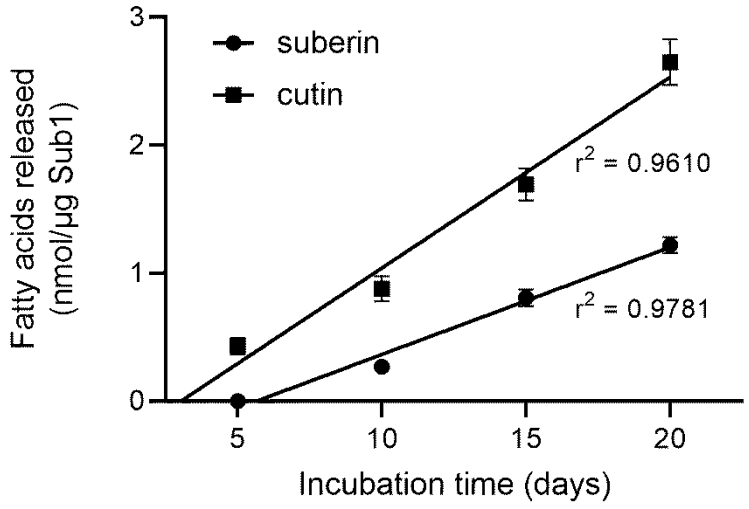

Figure 2.7. Degradation of cutin and suberin by enzyme Sub1 at room temperature over a 20-day period, as expressed by the release of fatty acids in the incubation medium. Data are the means ± SD of four replicates.

On the other hand, the release of TA showed that the Sub1 esterase also has the ability to hydrolyze the synthetic substrate PET. The addition of Triton X-100 to the

reaction mix enhanced the hydrolysis of PET by *ca*. 2.6-fold ($P < 0.0001$, *t*-test; Fig. 2.8A), after 10 and 15 days of incubation. The esterase activity of Sub1 on PET, in the presence of Triton X-100, increased over the incubation time as the amount of TA released during the degradation of PET was correlated with incubation time ($P < 0.0001$; Fig. 2.8B). Enzyme Sub1 showed high stability at 37°C over the test period (20 days) as the concentration of TA released in the incubation medium was linearly correlated the time ($r^2 = 0.9874$). However, the stability of Sub1 at 50°C rather fitted a non-linear curve ($r^2 = 0.9667$).

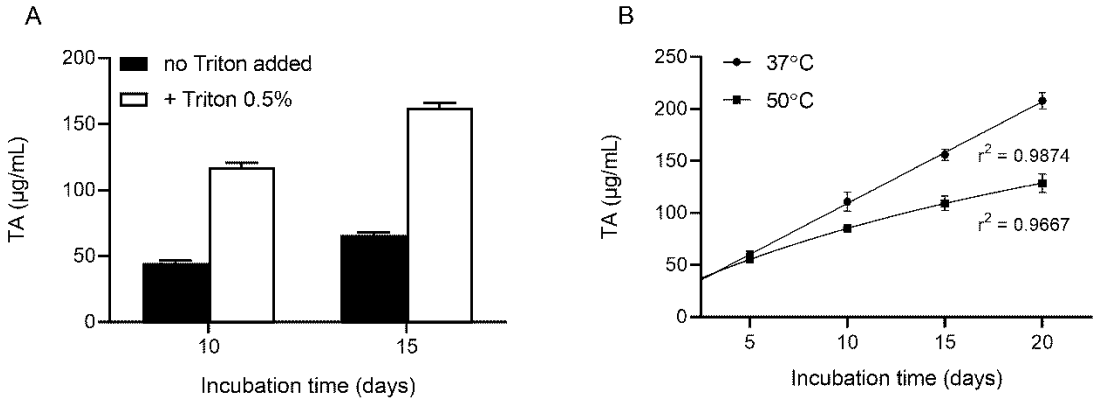

Figure 2.8. Concentrations of terephthalic acid (TA) released following the hydrolysis of ground particles of polyethylene terephthalate by 3 µg of Sub1 enzyme. (A) Effect of the presence of Triton X-100 (0.5%) on Sub1 performance after 10 and 15 days of incubation (at 37°C). (B) Sub1 enzymatic stability (in the presence of 0.5% Triton X-100) during 20 days of incubation at 37°C and 50°C. TA concentration was measured every 5 days. Data are the means ± SD of four replicates.

Discussion

Streptomyces scabies can colonize potato tuber surfaces and is able to directly penetrate potato cells (29). Several authors have proposed that the entry into potato tuber tissues may be facilitated by its production of esterases that degrade the suberin present in potato periderm (3, 23, 31). Although suberin degradation has been poorly studied, some fungal cutinases exhibit activity towards suberin (24). Protein Sub1 is part of the *S. scabies* secretome when grown in the presence of suberin, (2) and expression of the *sub1* gene is induced in the presence of suberin (23). This latter report predicted that Sub1 was a cutinase, due to its high sequence homology with other cutinases of fungal origin. The current work confirms this prediction, as we show that Sub1 is able to hydrolyze both cutin and suberin.

In this study, the heterologous production of the *S. scabies* Sub1 protein was successfully achieved in *E. coli*. Other studies have also reported the heterologous expression of bacterial esterases in *E. coli* (12, 36, 40). The molecular weight of the His-tagged Sub1 is estimated herein to be 25 kDa, which is consistent with the predicted molecular weight of mature Sub1 (18.7 kDa) plus the His-tag (4.9 kDa). The molecular weight of Sub1 thus appears to be less than that of most bacterial cutinases such as Tfu-0882 and Tfu-0883 from *Thermobifida fusca* (29 kDa; Chen et al. 2008), but is closer to those of fungal plant pathogen cutinases such as CcCUT1 from *Coprinopsis cinerea* (18.8 kDa; 24), and CutA from *Botrytis cinerea* (18 kDa; 42).

As seen with other cutinases, the purified Sub1 also has the ability to hydrolyze *p*-nitrophenyl esters. Sub1 was more active on *p*-nitrophenyl esters with short carbon chain, i.e. *p*-nitrophenyl butyrate (*p*-NPB, C4) and *p*-nitrophenyl octanoate (C8), compared to those with longer carbon chain (C10 and C12). Other microbial cutinases

were also reported to be more active on *p*-nitrophenyl esters harboring short fatty acid chains (24, 35). Using *p*-nitrophenyl butyrate as a substrate, the activity of Sub1 exhibited a typical Michaelis-Menten curve. The Sub1 enzyme showed affinity towards this substrate (K_m = 5.7 10^{-4} M), similar to two cutinases of *Fusarium solani* pv. *pisi* with K_m of 3.5 10^{-4} M and of 7.5 10^{-4} M, respectively (21). Although streptomycetes such as *S. scabies* and filamentous fungi belong to different kingdoms, they exhibit similar lifestyles and often share the same ecological niches (46). Their mycelia colonize various organic polymers and produce large amounts of extracellular enzymes to retrieve nutrients from these substrates. Similarity between Sub1 and fungal cutinases may thus reflect an adaptation to a similar lifestyle. Other *Streptomyces* extracellular enzymes, e.g. chitosanases from the GH75 family, are encoded by genes that are also mainly present in fungal and actinobacterial genomes (25).

Sub1 has a high similarity, at the amino acid level, with the cutinase CUT1 from *F. solani*, which is able to degrade suberin. Like CUT1, our findings indicated that Sub1 has a hydrolysis activity on suberin. However, Sub1 and CUT1 showed higher activity on cutin than on suberin, even though both *S. scabies* and *F. solani* infect and colonize potato tubers and are thus frequently in contact with potato suberin (17). This may indicate that the presence of aromatic compounds in suberin also contributes to its recalcitrant nature (15). It is however difficult to compare the efficacy of Sub1 with fungal cutinases since the methods used to monitor cutinase activity differ between studies (11, 24, 42, this work). To our knowledge, Sub1 represents the first bacterial cutinase for which an activity towards suberin has been demonstrated. Considering the ecological niche of this pathogen, that infects potato tubers, but not the aerial part of the plant, suberin most likely represents an important substrate for Sub1 in the environment and consequently, Sub1 can be designated as a suberinase. While the ability to degrade cutin has been shown to be important for the pathogenicity of various

fungal plant pathogens (16, 45), there is no evidence that the ability to degrade suberin is an asset in the infection process of plant pathogens. The Sub1 protein is unlikely to be an essential pathogenicity factor for *S. scabies* since the *sub1* gene has not been detected in other common scab-inducing *Streptomyces* species such as *S. acidiscabies* (23), and its primary benefit may involve the degradation of refractory polymers. However, it is plausible that Sub1 could confer an advantage to *S. scabies* over other common scab-inducing species by facilitating direct penetration of tubers, tuber colonization and its persistence in potato tuber debris.

While Sub1 is conserved in only a few streptomycetes, it presents high homology not only with some fungal cutinases but also with cutinase-like enzymes from animal pathogenic mycobacteria (23). These bacteria do not encounter cutin or suberin in their environment however, cutinases have been shown to be multifunctional enzymes that can act on phospholipids, polysorbates, triacylglycerols and triolein (32, 39). As multifunctional enzymes, cutinases are widely used in industry (9). In this work, the effect of Sub1 on PET, a synthetic polyester widely used for the production of textiles was tested. Sub1 was shown to be able to degrade PET as the quantity of terephthalic acid released from the synthetic polymer depended on the enzyme concentration and increased over the incubation time. The enzyme Sub1 maintained its activity for at least 20 days at 37°C, showing that Sub1 is stable, as has been demonstrated for other cutinases (13). Because of their functional properties, cutinases are considered as a link between esterases and lipases (10). The addition of a non-ionic surfactant such as Triton into a reaction mix usually promotes the activity of lipases but does not affect the activity of most cutinases. For example, the presence of Triton in the reaction mix increased the hydrolysis of polyester bis-(benzoyloxyethyl) terephthalate (3 PET) by a lipase secreted by *Thermomyces lanuginosus*, whereas it did not show any effect when the same substrate was exposed to cutinases secreted by *Thermobifida fusca* and *F. solani* (14). Triton increases the activity of lipases by promoting the opening of

a peptide lid located over the active site of the enzyme; such a lid is not present in cutinases (14). As observed with most cutinases, the addition of Triton X-100 did not affect cutin hydrolysis by Sub1 (data not shown). Nevertheless, the hydrolysis of *p*-nitrophenyl esters and of PET by Sub1 was improved in the presence of Triton.

In this work, it has been established that the *sub1* gene of *S. scabies* encodes a protein acting as a suberinase. The versatility of Sub1 can also be considered for its adoption in industrial applications. Recently, a cutinase-like enzyme drew global public attention. This enzyme, originally characterized in the bacterium *Ideonella sakaiensis* 201-F6, is able to degrade PET and has been reclassified as a PETase (47). Thereafter, Austin et al. (1) introduced modifications to the binding cleft of this enzyme and produced an engineered PETase with improved plastic-degrading properties, compared to the native enzyme. The current need for developing enzymes able to degrade refractory polymers of anthropic origin obviously makes further explorations of Sub1 capabilities relevant.

Acknowledgments

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CHAPTER 3

THE PLANT PATHOGENIC BACTERIUM *STREPTOMYCES SCABIES* DEGRADES THE AROMATIC COMPONENTS OF POTATO PERIDERM VIA THE β-KETOADIPATE PATHWAY

3.1. Preface

Suberin, a major component of potato periderm, composed of two main domains (polyaliphatic and polyaromatic). Previous research showed that the potato pathogen *S. scabies* can degrade the aliphatic part of suberin. However, the degradation of the aromatic part has not been documented. A previous proteomic analysis revealed the presence of proteins predicted to play a role in the degradation of aromatic compounds when *S. scabies* was grown in the presence of suberin suggesting the ability of this bacterium to degrade the aromatic moiety of suberin as well.

The results of this work showed for the first time the ability of *S. scabies* to degrade the two main constituents of the aromatic moiety of suberin, (the hydroxycinnamates; *trans*-ferulic and *p*-coumaric acids). The two compounds were shown to be degraded via the β-ketoadipate pathway based on proteomic analysis and a gene expression experiment. A *S. scabies* mutant was generated and was shown to be able to retrieve ferulic acid from suberin-enriched potato periderm.

The ability of *S. scabies* to utilize *trans*-ferulic and *p*-coumaric acids as carbon and energy sources may be advantageous in environments where they are limited. This

could provide an ecological advantage to *S. scabies* as a saprophyte in addition to an advantage as a pathogen as suberin degradation may facilitate the potato tuber colonization and the infection processes.

The results of this work are presented in Section 3.2 and submitted to the journal Frontiers in Microbiology, under the title "The plant pathogenic bacterium *Streptomyces scabies* degrades the aromatic components of potato periderm via the β-ketoadipate pathway". The authors of the manuscript are: Mario Khalil, Sylvain Lerat, Nathalie Beaudoin, Carole Beaulieu.

The contribution of each author in the manuscript is as follows: MK and CB designed the experiments. MK performed the lab work and analyzed the data with the help of SL who generated the mutant strain. MK designed the primers. MK and CB wrote the manuscript, with the critical review of CB, NB and SL. CB supervised the whole project. All authors approved the final version of the manuscript.

3.2. Manuscript of the article

Title: The plant pathogenic bacterium *Streptomyces scabies* degrades the aromatic components of potato periderm via the β-ketoadipate pathway.

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Abstract

The outer potato periderm layer consists of dead suberized cells. Suberin is a protective biopolymer made of a polyaliphatic domain covalently linked to polyaromatic moieties. Evidence accumulates that *Streptomyces scabies*, the main causal agent of potato common scab, can degrade the suberin aliphatic part but its ability to degrade the aromatic domain has not been documented. This polyaromatic domain is mainly composed of cinnamic acids. In this study, two cinnamates (*trans*-ferulic or *p*-coumaric acids) were added to the culture medium of *S. scabies* strains EF-35 and 87.22. HPLC quantification revealed that both strains efficiently utilized these compounds. A proteomic study coupled with gene expression analysis in *S. scabies* 87.22 led to the identification of putative catabolic pathways for cinnamates. Catabolism of both compounds appeared to occur via the β-ketoadipate pathway. Gene SCAB_15301, encoding for a putative vanillate monooxygenase, was partly deleted from *S. scabies* strain 87.22 genome. The mutant retained its ability to catabolize *trans*-ferulic acid into vanillate but lost its ability to further degrade the latter compound. When the wild-type mutant and complemented strains were grown in the presence of suberin-enriched potato periderm, accumulation of vanillic acid was observed only in the mutant culture medium. This work presents evidence that *S. scabies* can degrade not only the aliphatic part of suberin but also the constituents of suberin aromatic domain. This may provide ecological and pathological advantages to *S. scabies* as a saprophyte and pathogen.

Keywords: common scab, hydroxycinnamates, *trans*-ferulic acid, *Streptomyces scabiei*, suberin.

Introduction

Streptomyces scabies is the main causal agent of potato common scab, a potato disease which reduces both crop value and tuber marketability. This disease is characterized by corky dark colored lesions covering the tubers surface. The pathogenicity of *S. scabies* is mainly caused by the production of toxins known as thaxtomins, which induce cell death in plant cells and tissues (Duval et al., 2005; Goyer et al., 1998; Healy et al., 2000).

The potato skin contains high levels of suberin, a biopolymer playing a protective role against pathogens entry in potato tuber tissues. Suberin is a complex polymer consisting of two covalently-linked domains (Bernards, 2002). The polyaliphatic domain located between the plasma membrane and the primary cell wall consists of esterified long-chain fatty acids. Evidence accumulates that *S. scabies* produces esterases degrading this aliphatic part (Beaulieu et al., 2016). A secretome analysis of *S. scabies* cultures grown in the presence of suberin was performed to identify the enzymes potentially involved in the degradation of suberin (Komeil et al., 2014). Glycosyl hydrolases were the most abundant proteins in the supernatant of suberin-containing medium, in addition to several enzymes involved in lipid metabolism (Komeil et al., 2014).

The polyaromatic domain of suberin is a lignin-like structure embedded in the primary cell wall. It is mainly composed of polyhydroxycinnamates (Bernards and Lewis, 1998; Bernards et al., 1995), where *trans*-ferulic and *p*-coumaric acids figure among the main hydroxycinnamates forming the aromatic moiety of suberin (Riley and Kolattukudy, 1975). Previous work has shown the importance of *trans*-ferulic acid in

keeping the integrity of both suberin and periderm wax (Serra et al., 2010), therefore preventing pathogen entry.

Suberin degradation, especially of the aromatic part, has been mostly investigated in fungi. As an example, *Aspergillus nidulans* was reported to degrade the aromatic portion of suberin by monitoring the degradation products when *A. nidulans* was grown on suberized cell walls (Martins et al., 2014). The microbial degradation of suberin is a process that has been poorly characterized. However, there are several *Streptomyces* species that have been reported to degrade aromatic compounds. *Streptomyces* sp. strain ERI-CPDA-1, isolated from oil contaminated soil, was able to degrade petroleum and polycyclic aromatic hydrocarbons (PAHs). The degradation products detected were benzaldehyde, catechol, phenylacetic acid and protocatechuic acid (Balachandran et al., 2012). *Streptomyces setonii* strain ATCC 39116, a thermophilic soil actinomycete, was also shown to be able to degrade single aromatic compounds, including phenol and benzoate, through the ortho-cleavage pathway (Park and Kim, 2003).

A previous study showed that in the presence of suberin, *S. scabies* secreted the extracellular protein C9Z2P6, a putative 3-oxo-5,6-dehydrosuberyl-CoA semialdehyde dehydrogenase, which might be involved in the degradation of aromatic compounds. Moreover, the gene encoding this protein (SCAB_57301) was overexpressed in presence of suberin (Komeil et al., 2014), suggesting that *S. scabies* can degrade the aromatic part of suberin.

In the present study, the ability of *S. scabies* to degrade the main aromatic constituents of the potato suberin, *trans*-ferulic acid and *p*-coumaric acid, is investigated. A

hypothetical degradation pathway of *trans*-ferulic and *p*-coumaric acids in *S. scabies* 87.22 is proposed.

Materials and methods

Culture conditions

Strains and plasmids used in this study are listed in Table 3.1. Inocula of *Streptomyces scabies* strains EF-35 and 87.22 were prepared by inoculating approximately 10^8 spores in 50 mL of yeast malt extract (4 g L^{-1} glucose, 4 g L^{-1} yeast extract, and 10 g L^{-1} malt extract). The bacteria were then incubated at 30°C with shaking (250 rpm) for 48 h followed by bacterial cells recovery using centrifugation (3,500×*g*) for 10 min. The collected pellets were washed two times and resuspended in 5 volumes of saline solution (NaCl 0.85%) and the resulting bacterial suspensions were used as inocula in further experiments. These bacterial inocula (100 µL) were added to 50 mL of the control medium (CM) composed of 0.5 g L^{-1} l-asparagine, 0.5 g L^{-1} K_2HPO_4, 0.2 g L^{-1} $MgSO_4 \cdot 7H_2O$, 10 mg L^{-1} $FeSO_4 \cdot 7H_2O$, and 0.05% (w/v) casein hydrolysate. This medium could be supplemented with *trans*-ferulic acid (60 µM), *p*-coumaric acid (50 µM), vanillic acid (60 µM), protocatechuate (60 µM), suberin 0.1% (w/v) or a combination of both ferulic and vanillic acids. The minimum inhibitory concentrations (MIC) of hydroxycinnamates in *S. scabies* strains EF-35 and 87.22 were determined and the used concentrations in this study were selected to be in the subinhibitory levels. Suberin was purified from potato tubers according to Kolattukudy and Agrawal (1974) with modifications proposed by Komeil et al. (2013). Culture media were adjusted to pH 7.0 and incubated with shaking (250 rpm) at 30°C. *Escherichia coli* strains were grown on LB agar plates at 37°C. When necessary, spectinomycin (100 µg mL^{-1} final concentration) was added to the culture medium.

Table 3.1. Bacterial strains and plasmids used in this study.

Strain or plasmid	Description	Reference
Strains		
Streptomyces scabies EF-35	Wild strain	Faucher et al. (1992)
S. scabies 87.22	Wild strain	Loria et al. (1995)
S. scabies ΔSCAB_15301	*S. scabies* 87.22 carrying a non-functional SCAB_15301 gene	This work
S. scabies com15301	ΔSCAB_15301 complemented with a functional SCAB_15301 gene carried by pSET15301	This work
Escherichia coli NEB® 5-alpha	High-efficiency competent strain	New England Biolabs
E. coli ET12567	Non-methylating conjugation strain	MacNeil et al. (1992)
Plasmids		
pUZ8002	Non-transmissible RP4 derivative plasmid	Kieser et al. (2000)
pCRISPomyces-2	Plasmid for targeted genome editing in *Streptomyces* species	Cobb et al. (2015)
pCRISPo-proto15301	The pCRISPomyces-2 plasmid containing a SCAB_15301-specific sequence that the Cas9 system can target	This study
pCRISPo-15301-full	The pCRISPo-proto15301 plasmid with a partially deleted SCAB_15301 gene inserted in a unique *Xba*I site	This study
pSET152m	Modified pSET152 vector in which the apramycin resistance gene *aac(3)IV* is replaced with the spectinomycin resistance gene *aadA*	Lu et al. (2007)
pSET15301	pSET152m derivative carrying SCAB_15301 gene	This study

Quantitation of cinnamic acids in *Streptomyces scabies* culture broths

Amounts of *trans*-ferulic, *p*-coumaric and vanillic acids in culture broths were quantified by high performance liquid chromatography (HPLC). 500 µL of each culture was sampled each three hours. The collected samples were filtered through 0.2 µm syringe filters prior to injection in the device. Cinnamic acids were detected using an Agilent 1260 Infinity high-performance liquid chromatograph equipped with a reverse-phase C18 column with a particle size of 5 µm. They were eluted using a 25 to 75% acetonitrile linear gradient for 10 min at a flow rate of 1 mL min^{-1} and monitored at 310 nm (*trans*-ferulic and *p*-coumaric acids) or 254 nm (vanillic acid). Standard curves were established for each compound analyzed using dilutions of known quantity of the compounds. The experiment was done in three biological replicates and was repeated twice.

Proteomics analysis

Streptomyces scabies strain 87.22 was selected for further experiments since its complete genome was sequenced and will be suitable for genetic manipulation. Also, ferulic acid was chosen for the proteomic analysis because of its important role in keeping the integrity of the suberin structure. *Streptomyces scabies* strain 87.22 was grown in CM for 4 days. Six and 12 h later, *trans*-ferulic acid (60 µM) was then added or not to the culture medium and the culture was incubated for an additional 6 h. Bacterial cells were then collected by centrifugation at 3,500×*g* for 10 min and were washed with phosphate buffered saline (PBS). PBS was removed by centrifugation and the intracellular proteins of collected cells were extracted by adding PBS and freezing the cells in ethanol kept at –80°C. The cells were thawed for 10 min at room temperature. This freeze-thaw lysis was repeated twice, and the suspension was

sonicated for 10 s. The suspension was centrifuged at 4°C for 10 min (3,500×*g*) and supernatant containing the proteins was kept at 4°C. Intracellular proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis [10% (w/v) SDS-PAGE] and in-gel protein digestion were carried out according to Komeil et al. (2013). Mass spectrometry was conducted at the Proteomics Platform of the Quebec Genomics Center (Quebec City, Canada) using a hybrid quadrupole time-of-flight (QqTOF) (TripleTOF 5600 plus, SCIEX) coupled to a capillary HPLC for peptide separation via a nanospray ionization source. All MS/MS spectra were then interpreted using Mascot (Matrix Science, London, UK) to search *S. scabies* strain 87.22 Uniref100 database to provide statistically validated matches between observed spectra and identified peptides and list of proteins. The results were then uploaded to the scaffold software program (version Scaffold 4.8.8, Proteome Software, Portland, OR, USA) and a filter was set with a 95% minimum protein ID probability with a minimum number of two unique peptides, in which the cut-offs for peptide thresholds were set to 1.0% false discovery rate (FDR). Protein function was predicted using the UniProt, NCBI, KEGG, and COG databases. The normalized spectral count (NSpC) of proteins was obtained by dividing the number of spectra (SpC) for a protein by the molecular weight (MW) of the corresponding protein (Neilson et al., 2011). Two replicates for each treatment were done.

qRT-PCR

Following the proteome analysis which identified some putative targets, the expression of the potential cinnamic acid catabolic genes was determined in the presence of ferulate or coumarate as follows. *S. scabies* strain 87.22 was grown in CM for 4 days and then *trans*-ferulic or *p*-coumaric acid was added or not to the culture medium. Three hours later, 10 mL of each culture medium was mixed with 2 mL of stop solution (ethanol/acidic phenol, 95:5, [v/v]) to prevent RNA degradation (Joshi et

al., 2007). Bacterial cell pellets were recovered by centrifugation at 4°C for 10 min at 3,500×*g* and stored at –80°C until further use. RNA was extracted from cells using the RNeasy Mini kit (Qiagen) (Lerat et al., 2010). Two µg of total isolated RNA were then reverse transcribed to cDNA using the Maxima First strand cDNA synthesis kit (ThermoFisher Scientific) with random primers according to the manufacturer's instructions. Diluted cDNA (10×) was used to perform quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) using Mx3000P qPCR system (Agilent Technologies) with the BrightGreen 2x qPCR MasterMix-Low ROX. The q-RT PCR conditions were 95°C for 10 min followed by 35 cycles at 95°C for 15 s and 60°C for 30 s. Relative expression levels were determined by using the comparative C_T values according to Pfaffl (2001) with *gyrA* gene as a reference gene which was previously validated as a stable reference gene. The primers were designed, and their sequences are illustrated in Table 3.2. The experiment was done in three biological replicates with two technical replicates per sample and was repeated twice.

Table 3.2. Primers used in the gene expression assay.

Gene assignation	Predicted function of the corresponding protein	Primer sets (5′ - 3′)
SCAB_13631	Protocatechuate-3,4-dioxygenase beta subunit	For:CACATCCACTTCTCGCTCTT Rev:ACTGGATGATCGGGTCGTA
SCAB_13661	3-oxoadipate enol-lactone hydrolase	For:CGCTTCCAGGACTTCATCTC Rev:CATGGCCAGTTCGTCGTAG
SCAB_15301	Vanillate monooxygenase alpha subunit	For:CAACCACACGGTCGTCAT Rev:GATGTTGATGCTCAGCTCCT
SCAB_15321	Iron-sulfur oxidoreductase beta subunit	For:CTACGTGCACACGGAGTT Rev:GGTTCCAGGGCGAAGTT
SCAB_15331	IclR-family transcriptional regulator	For:GAGCATCCCGCACTGAC Rev:CGAGCGTGAGCAGGAAG
SCAB_15591	Feruloyl-CoA hydratase	For:CACCCTGTCGCTGTTCAT Rev:CCTTGTTGAGGCCGTAGTT
SCAB_15601	Feruloyl-CoA synthetase	For:GACGACACCTGCATCATCA Rev:GTTGACGGCGTTCCAGAT
SCAB_2141	2,3-dihydroxy-2,3-dihydro-phenylpropionate dehydrogenase	For:GACGAGATCTTCGCGATCAA Rev:GCGTTGGACAGGGTCAT
SCAB_45751	Gyrase A (*gyrA*)	For:GCCATCAACCTCCGTGAAA Rev:CGGATCGATTGTGCCTTCTT
SCAB_6611	3-oxoadipate CoA-transferase subunit	For:CTACGTGATGATGACGCTCTT Rev:ACCGTGGTCGGTGTAGA
SCAB_6621	3-oxoadipate CoA-transferase subunit A	For:CTCACCATCGTCTCCAACAA Rev:AAGGAGCAGAGCACCTTG

Deletion of the putative vanillate monooxygenase gene SCAB_15301 from *Streptomyces scabies* 87.22 genome

Streptomyces scabies strain 87.22 carrying a non-functional SCAB_15301 gene was constructed using the CRISPR-Cas9 system. The gene was selected to investigate the ability of *S. scabies* 87.22. to further degrade vanillate as it was detected in a previous proteomic analysis in presence of ferulic acid. The procedure of Wang et al. (2016), specifically developed for *Streptomyces* species, was followed with plasmid pCRISPomyces-2 (Addgene) to carry the construction.

Briefly, a short DNA sequence, unique to SCAB_15301 in the *S. scabies* genome, was introduced into pCRISPomyces-2, generating plasmid pCRISPo-proto15301. A partially deleted version of gene SCAB_15301 (generating a stop codon) was then created, using the Gibson Assembly Master Mix (New England Biolabs), and inserted in pCRISPo-proto15301. This region serves as repair template to the recombination process that follows the disruption of target DNA by the CRISPR-Cas9 system. The obtained plasmid was named pCRISPo-15301-full.

pCRISPo-15301-full was introduced in the *Escherichia coli* conjugation strain ET12567-pUZ8002. Conjugation between *S. scabies* 87.22 and *E. coli* ET12567-pUZ8002 carrying plasmid pCRISPo-15301-full was performed as described in Kieser et al. (2000). The insertion of non-functional SCAB_15301 gene in the genome of exconjugants was confirmed by DNA sequencing using primers annealing slightly upstream and downstream of the editing template sequence. Finally, the plasmid carrying the pCRISPomyces system was cleared from the *S. scabies* recombinant strain after three serial cultures in ISP-2 medium (Wang et al., 2016).

Complementation of ΔSCAB_15301 deletion mutant

Genomic DNA was obtained from *S. scabies* strain 87.22 using the salting-out procedure (Kieser et al., 2000). SCAB_15301 along with the upstream region (1799 bp) were amplified from the wild type strain using two primers to which the restriction sequences of the digestion enzymes were added (5'-CGGAATTCCGTCTCCTCCTCGGTCCTCACCC-3' and 5'-GCTCTAGAGCTCACAGGACCTTCTCCACCGG-3'). PCR reactions were carried out in a final volume of 50 µL containing 1 µL (233 ng μL^{-1}) of template DNA, 2.5 µL (10 µM) of each primer, 10 µL of 10× buffer, 1 µL of 10 mM dNTPs, 0.5 µL of Q5® high-fidelity DNA polymerase, 10 µL of Q5 high GC enhancer. The PCR cycling conditions were as follows: an initial pre-denaturing step at 98°C for 30 s, 35 cycles at 98°C for 10 s, 72°C for 30 s, 72°C for 30 s and a final extension step at 72°C for 2 min using Bio-Rad T100 thermal Cycler.

Amplicons of the putative vanillate monooxygenase gene SCAB_15301 and integrative vector pSET152m (Lu et al., 2007) were both digested with EcoRI and XbaI at 37°C for 2 h. SCAB_15301 gene was ligated into the digested plasmid using DNA ligase at 16°C for 2 h. The resulting plasmid pSET15301 was transformed to *E. coli* ET12567-pUZ8002. The transformation was done as described by Kieser et al. (2000). The conjugation between *E. coli* ET12567-pUZ8002 pSET15301 and *S. scabies* ΔSCAB_15301 was carried out as proposed by Kieser et al. (2000). The complementation was confirmed by sequencing the inserted gene SCAB_15301 using the same primers used for SCAB_15301 amplification. Sequencing was carried out at Genome Sequencing and Genotyping Platform (Quebec City, Canada) using Sanger technique.

Results

Utilization of cinnamic acids by *Streptomyces scabies*

A time course study of both *trans*-ferulic and *p*-coumaric acids depletion was carried out by sampling the *S. scabies* culture at 3-h intervals for 15 h. Both *S. scabies* strains EF-35 and 87.22 gradually utilized these compounds. After 15 h of incubation, most of *trans*-ferulic and *p*-coumaric acids were consumed (Fig. 3.1. A and 3.1. B). During the first hours of incubation, both tested strains showed higher degradation affinity towards *trans*-ferulic acid when compared to *p*-coumaric acid, and especially *S. scabies* 87.22 which consumed about 84% of the added *trans*-ferulic acid after 6 h of inoculation (Fig. 3.1. B).

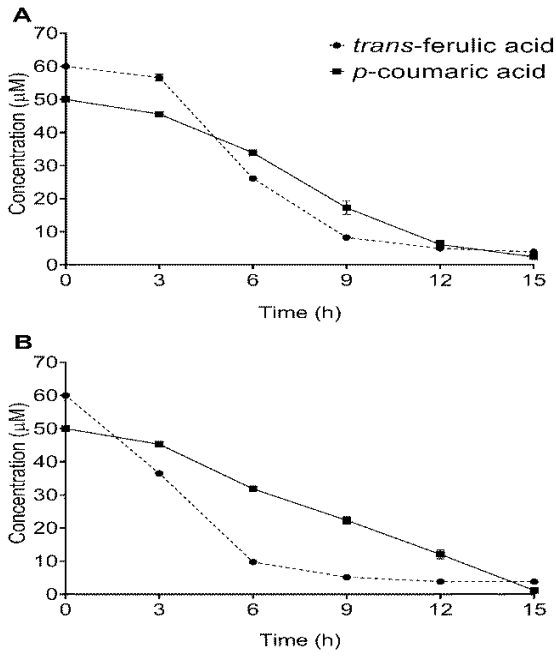

Figure 3.1. Kinetics of *trans*-ferulic and *p*-coumaric acids utilization in *Streptomyces scabies* strains EF-35 (A) and 87.22 (B). Data shown are the mean of three biological replicates (± SD).

SCAB_15301 has been identified in *S. scabies* 87.22 genome (using basic local alignment search tool [BLAST]) as a gene encoding a vanillate monooxygenase which is responsible for bioconversion of vanillic acid into protocatechuate. SCAB_15301 was selected to test the ability of *S. scabies* 87.22 to further degrade vanillate. To confirm the predicted function of this gene, SCAB_15301 was deleted from *S. scabies* 87.22 genome using CRISPR/Cas9-based method. The CRISPR/Cas9-based method has been shown to be effective in *Streptomyces* genome editing (Wang et al., 2016). However, the success rate in *S. scabies* using the CRISPR/Cas9-based system was very low as only one exconjugant was obtained and all efforts to obtain other mutants failed. Nonetheless, depletion of ferulic acid by the deletion mutant *S. scabies* ∆SCAB_15301 was observed. This mutant was able to convert over 80% of the initial amount of ferulic acid into vanillate within 9 h (Fig. 3.2. C). This contrasted with both the wild and complemented strains, where vanillate did not accumulate into the culture medium (Fig. 3.2. A and 3.2. B). Moreover, when vanillic acid was added to the culture medium of strains 87.22, ∆SCAB_15301 and *S. scabies* com15301, catabolism of vanillate was observed in strain 87.22 and the complemented strain but not in ∆SCAB_15301 (Fig. 3.3.), indicating that SCAB_15301 effectively codes for a putative vanillate monooxygenase.

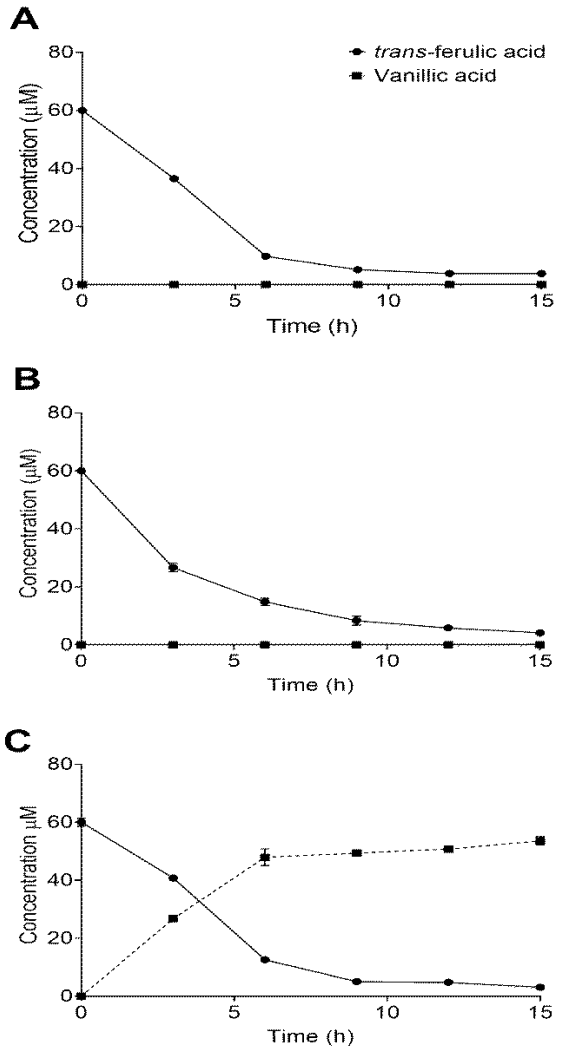

Figure 3.2. Quantitation of the utilization of *trans*-ferulic acid and production of vanillic acid over time in the presence of (A) *S. scabies* 87.22. (B) *S. scabies* com15301. (C) ΔSCAB_15301. Data shown are the mean of three biological replicates (± SD).

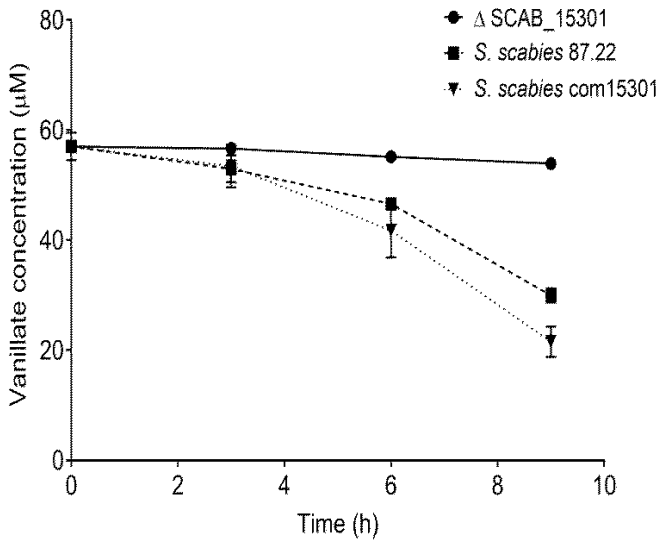

Figure 3.3. Catabolism of vanillate in *Streptomyces scabies* strains 87.22, ΔSCAB_15301 and com15301. Data shown are the mean of three biological replicates (± SD).

Effect of *trans*-ferulic acid on the *Streptomyces scabies* 87.22 proteome

Intracellular proteins produced by *S. scabies* 87.22 during growth in CM supplemented or not with *trans*-ferulic acid are listed in table S1 (Supplementary Table). Sixty-nine proteins were found exclusively in the presence of *trans*-ferulic acid. These proteins were categorized according to their functional groups (Table 3.3.). Among them, seven proteins were identified to be potentially involved in the degradation of aromatic compounds (Table 3.3.) and five were included in the stress mechanism functional group. Only two proteins were detected exclusively in CM without *trans*-ferulic acid; a transmembrane efflux protein (C9Z0L4) with normalized spectral count (NSpC) of 0.03 and a membrane protein with unknown function with NSpC of 0.04.

Table 3.3. Proteins detected only in *Streptomyces scabies* 87.22 proteome in control medium supplemented with ferulic acid.

Uniprot accession #	Corresponding gene in *S. scabies* 87.22	Predicted function	Abundance (NSpC)
Degradation of aromatic compounds			
C9ZBH8	SCAB_15301	Vanillate monooxygenase	0.10
C9YX73	SCAB_6611	3-oxoadipate CoA-transferase subunit	0.12
C9YX74	SCAB_6621	3-oxoadipate CoA-transferase subunit A	0.16
C9ZBI0	SCAB_15321	Iron-sulfur oxidoreductase beta subunit	0.08
C9ZBK6	SCAB_15591	Enoyl-CoA hydratase	0.18
C9ZBK7	SCAB_15601	Feruloyl-CoA synthetase	0.05
C9Z892	SCAB_13631	Protocatechuate 3,4-dioxygenase beta subunit	0.10
Carbohydrate transport and metabolism			
C9Z6Y3	SCAB_28241	Pyruvate kinase	0.11
C9ZGJ3	SCAB_18361	Malate synthase	0.11
C9Z376	SCAB_86561	Alpha-L-rhamnosidase	0.02
C9Z510	SCAB_11461	Succinate dehydrogenase	0.11
C9ZF59	SCAB_33461	Polyphosphate glucokinase	0.07
C9YZG5	SCAB_54911	UDP-glucose 6-dehydrogenase	0.04
Stress mechanism			
C9Z1S1	SCAB_9141	Betaine aldehyde dehydrogenase	0.34
C9Z9F8	SCAB_76571	Uracil-DNA glycosylase	0.12
C9YZC4	SCAB_39071	Hydroxymethylbilane synthase	0.05
C9ZDL6	SCAB_47371	Type II toxin-antitoxin	0.18
C9YX55	SCAB_83581	Aspartate ammonia-lyase	0.04
Lipid metabolism			
C9ZBK6	SCAB_15591	MaoC family dehydratase	0.18
C9ZBK7	SCAB_15601	Fatty-acyl-CoA synthase	0.05

C9Z9V3	SCAB_0081	Hydrolase	0.05
C9YVX2	SCAB_20961	Fatty acid oxidation complex	0.11
Transcriptional, translational, ribosomal structure and biogenesis			
C9Z4I3	SCAB_74011	Phenylalanine tRNA ligase	0.06
C9Z626	SCAB_74721	Exonuclease	0.15
C9Z1C5	SCAB_72001	GTPase Der	0.02
C9Z0Y8	SCAB_55691	Peptide chain release factor 2	0.07
C9ZAL3	SCAB_45881	Methyltransferase G	0.07
C9Z669	SCAB_75161	Transcription antitermination protein	0.12
C9Z0W5	SCAB_39991	50S ribosomal protein L31 type B	0.20
C9YUZ3	SCAB_36381	Transcriptional regulator	0.05
C9Z1E8	SCAB_72241	Transcriptional repressor	0.07
C9Z633	SCAB_74791	Elongation factor	0.02
Signal transduction mechanism			
C9Z568	SCAB_26741	Histidine kinase	0.01
C9ZGN5	SCAB_33821	Phosphorelay signal transduction pilus	0.03
Nucleotide transport and metabolism			
C9ZF48	SCAB_33351	Nucleotide modification protein	0.08
C9Z0P9	SCAB_39321	Phosphatase	0.06
C9ZGK9	SCAB_18521	Uricase	0.05
Cell wall/membrane/envelope biogenesis			
C9YVQ7	SCAB_6101	Conserved protein (MreB, spore wall)	0.07
Coenzyme transport and metabolism			
C9Z245	SCAB_25301	Riboflavin biosynthesis protein	0.05
C9Z8W6	SCAB_45281	Guanylyltransferase	0.06
C9Z1Y6	SCAB_9791	Precorrin-8X methylmutase	0.08
Amino acid transport and metabolism			
C9Z5A1	SCAB_27071	Acetolactate synthase small subunit	0.10

C9ZGY7	SCAB_49631	Sulfurtransferase	0.09
C9Z6A7	SCAB_88411	Dihydroxy-acid dehydratase	0.03
C9YZD9	proC	Pyrroline-5-carboxylate reductase	0.06
C9ZBN4	SCAB_15871	Oxidoreductase	0.05
C9Z8G8	SCAB_28961	Homoserine dehydrogenase	0.04
C9ZBK8	SCAB_15611	Amidohydrolase	0.12
Transport, secretion and efflux			
C9ZC07	SCAB_46421	ABC transporter (cytochrome bd)	0.01
C9YX17	SCAB_83191	Transport system integral protein	0.05
C9ZGM4	SCAB_18691	Cation transport protein (Mg)	0.07
C9ZAS1	SCAB_61981	Metal-binding lipoprotein (Zn)	0.05
General function prediction only			
C9ZBK9	SCAB_15621	Dehydrogenase / reductase	0.27
C9ZCN6	SCAB_78601	Ligase	0.03
C9ZE91	SCAB_79211	RarE (Conservon) homologue	0.04
C9Z4V2	SCAB_87941	Hydrolase	0.09
C9YU23	SCAB_81961	Ligase and argininosuccinate lyase	0.06
C9Z2U3	SCAB_72631	Aldehyde dehydrogenase	0.04
C9Z7E5	SCAB_59891	Serine/threonine-protein phosphatase	0.04
C9ZAA1	SCAB_29911	Putative methyltransferase	0.07
Unknown function			
C9Z8Y5	SCAB_60551		0.15
C9ZH42	SCAB_50211		0.11
C9Z8X7	SCAB_60471		0.13
C9Z0T5	SCAB_39691		0.03
C9ZDU8	SCAB_48211		0.06
C9YZT7	SCAB_71141		0.06
C9YTB7	SCAB_35231		0.11

C9YZA8	SCAB_38901	0.05
C9Z0S1	SCAB_39541	0.33

NSpC: Calculated by dividing the number of spectra (SpC) for a protein by the molecular weight (MW) of the corresponding protein

Effects of hydroxycinnamates on the expression of the putative aromatic compounds degradation genes

The expression of seven genes associated with the degradation of aromatic compounds functional group (Table 3.3.) and of three additional genes that were identified using basic local alignment search tool (BLAST) from *S. scabies* 87.22 genome was investigated. The three additional genes were SCAB_15331 (a putative IclR-family transcriptional regulator gene located in the vicinity of SCAB_15301 and SCAB_15321), SCAB_2141 (2,3-dihydroxy-2,3-dihydro-phenylpropionate dehydrogenase encoding gene) and SCAB_13661 (3-oxoadipate enol-lactone hydrolase-encoding gene). All genes tested were overexpressed (P<0.05, LSD test) in the presence of at least one of the two cinnamic acids (*trans*-ferulic acid or *p*-coumaric acid) (Fig. 3.4.).

SCAB_15601 and SCAB_15591 code for a putative feruloyl-CoA synthetase and a putative enoyl-CoA hydratase, respectively. They are potentially responsible for the transformation of ferulic acid into vanillic acid. Their gene expression was increased over 100- and 300-fold in the presence of ferulic and coumaric acids, respectively (Fig. 3.4.). The expression of the three genes identified as potential candidates for the conversion of vanillate into protocatechuate showed between 15-fold (SCAB_15331) and 300-fold overexpression (SCAB_15301 and SCAB_15321) in the presence of *trans*-ferulic acid (Fig. 3.4.). In contrast, the relative expression of these three genes

was not statistically different when the bacteria were grown in the control medium supplemented or not with *p*-coumaric acid.

SCAB_2141 expression was found to be induced by *p*-coumaric acid but not by *trans*-ferulic acid. SCAB_2141 showed high expression in the presence of *p*-coumarate (20-fold upregulated). Whereas, no difference was recorded when *trans*-ferulic acid was added to the control medium (Fig. 3.4.).

The four genes involved in the main β-ketoadipate pathway that were tested showed very similar expression patterns towards both substrates. SCAB_13631 showed a relative expression increasing from 40 and 60-fold in the presence of *p*-coumaric and *trans*-ferulic acid, respectively. SCAB_13661, a gene predicted to be responsible for conversion of γ-carboxymuconolactone into β-ketoadipate enol-lactone, showed a 36-fold overexpression when the control medium was supplemented with *trans*-ferulic acid and a 27-fold overexpression with *p*-coumarate. SCAB_6611 showed higher expression in the presence of *trans*-ferulic acid when compared to the presence of *p*-coumaric acid. SCAB_6621, which is predicted to further metabolize β-ketoadipate into β-ketoadipyl-CoA, showed around 30-fold overexpression with both substrates when compared to the control medium. A hypothetical degradation pathway of *trans*-ferulic and *p*-coumaric acids is proposed based on the results from the proteomic analysis and the gene expression experiment (Fig. 3.5.).

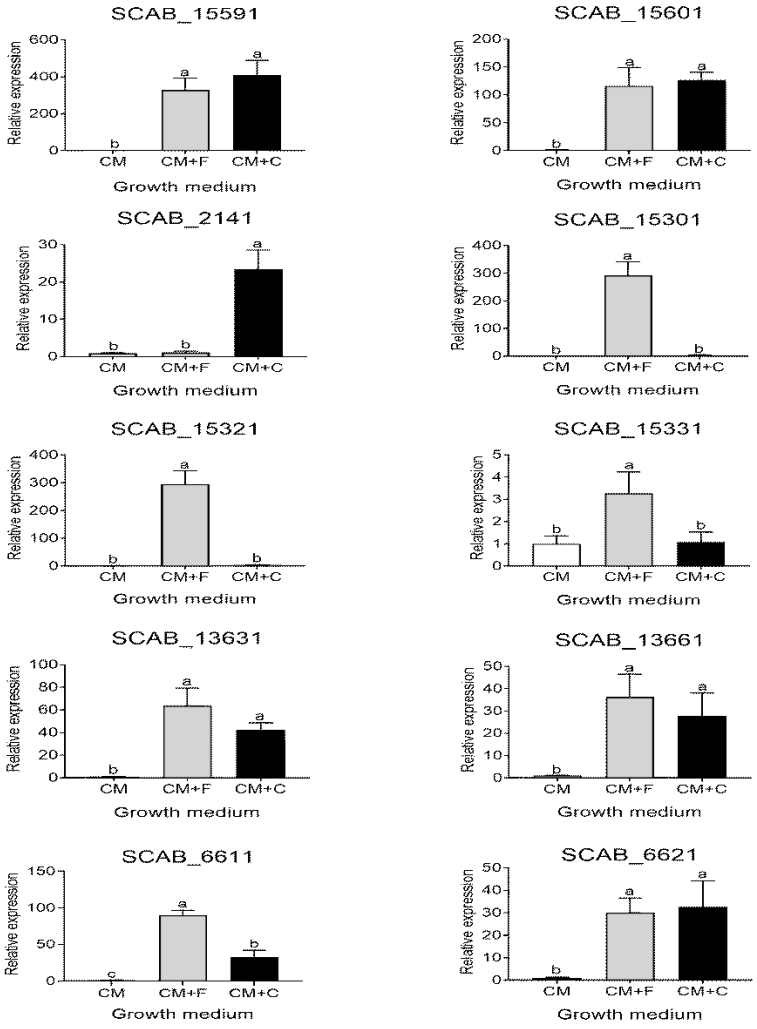

Figure 3.4. Relative expression levels (Error bars represent standard deviations) of targeted genes involved in *trans*-ferulic and *p*-coumaric acids degradation from *Streptomyces scabies* 87.22 grown in control medium (CM) alone, CM supplemented with *trans*-ferulic acid (CM+F, gray bars) and CM supplemented with *p*-coumaric acid (CM+C, black bars). Data were normalized with the *gyrA* gene which was used as an internal control. Data shown are the mean of three biological replicates ($P<0.05$, LSD test).

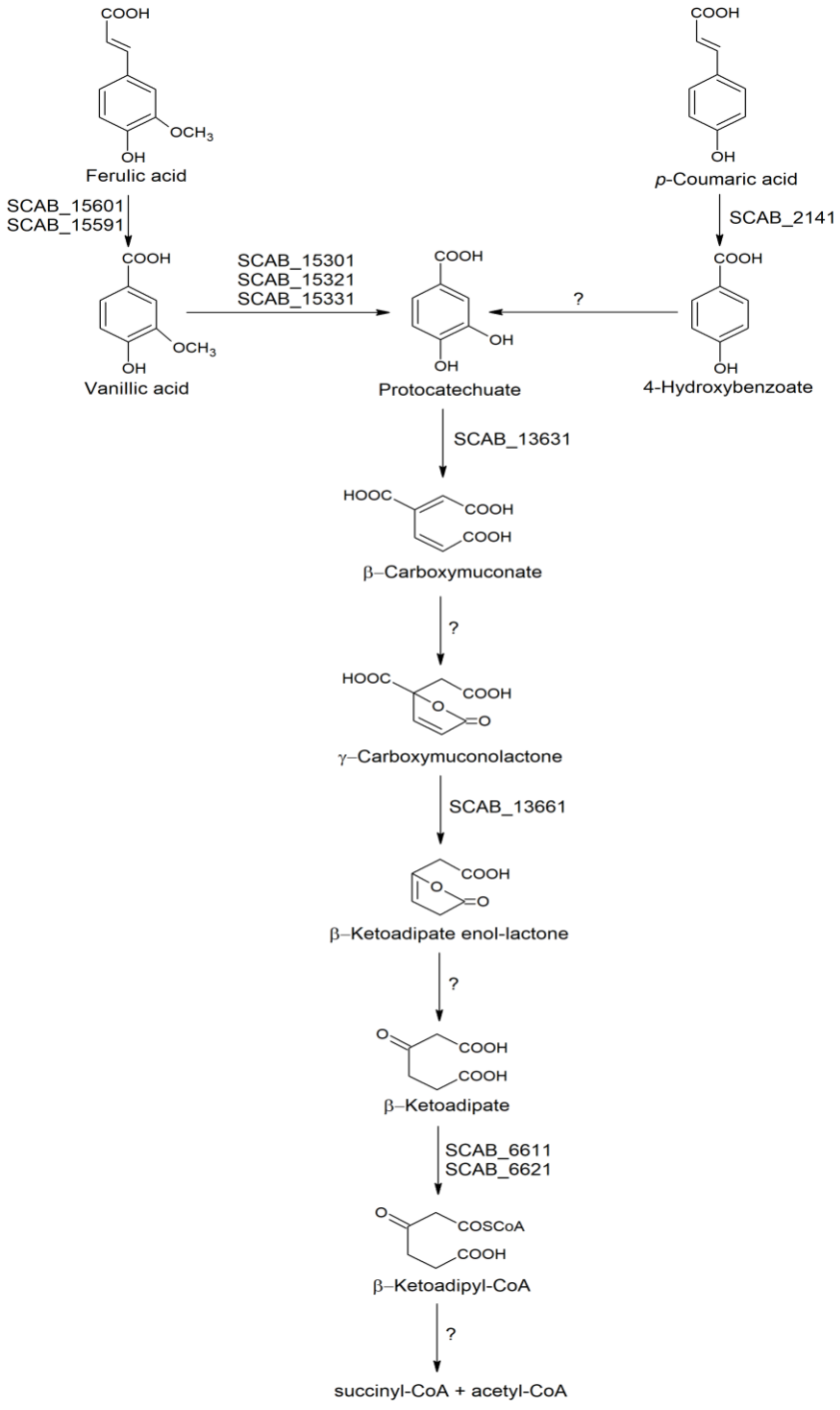

Figure 3.5. Hypothetic degradation pathway of *trans*-ferulic and *p*-coumaric acids in *Strepotmyces scabies* 87.22.

To determine if *trans*-ferulic or *p*-coumaric acid or one of their degradation products were the inducers of the β-ketoadipate pathway, the expression of SCAB_13631 (the first gene in the proposed β-ketoadipate pathway) was tested in presence of *trans*-ferulic, *p*-coumaric or protocatechuic acids. The gene SCAB_13631 showed around 60-fold overexpression in the presence of *trans*-ferulic acid in the wild strain. Addition of *trans*-ferulic acid in the culture medium of the deletion mutant strain ΔSCAB_15301 did not significantly induce transcription of gene SCAB_13631 (relative expression of 1.1 ± 0.31) since the protein from SCAB_15301 gene acts upstream of SCAB_13631. SCAB_13631 showed similar expression rate in the presence of coumarate (42.36 ± 4.18 and 44.3 ± 3.9, respectively) or protocatechuate (4.05 ± 0.38 and 4.23 ± 0.97, respectively) in the wild strain and the deletion mutant.

Effects of suberin on the expression of the putative aromatic compounds degradation genes

The expression of the genes predicted to be involved in the degradation of aromatic compounds (see above) was tested in the presence of suberin-enriched potato periderm. All tested genes, except gene SCAB_6621, showed a higher level of expression when *S. scabies* 87.22 was grown in the presence of suberin (between 2- to 30-fold overexpression) (Fig. 3.6.).

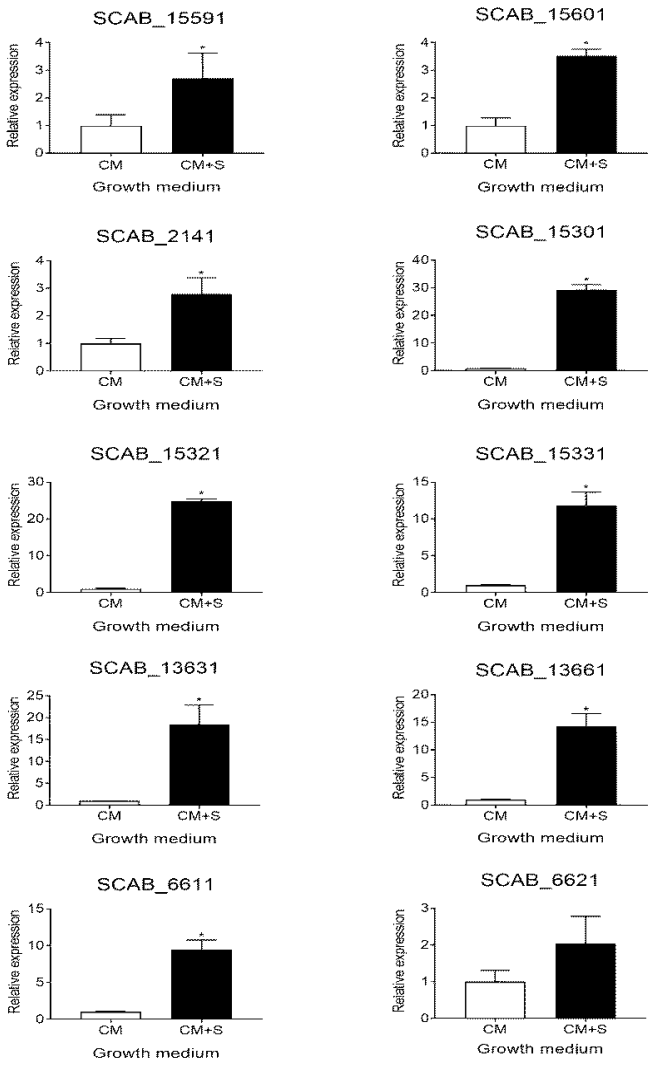

Figure 3.6. Relative expression levels (Error bars represent standard deviations) of targeted genes involved in *trans*-ferulic and *p*-coumaric acids degradation from *S. scabies* 87.22 grown in control medium (CM) alone and control medium (CM) supplemented with suberin (CM+S, black bars). Data were normalized with the *gyrA* gene which was used as an internal control. Data shown are the mean of three biological replicates ($P<0.05$, *t*-test).

Vanillate accumulation in the mutant culture media

When the wild type strain *S. scabies* 87.22, deletion mutant ΔSCAB_15301 and complemented strain *S. scabies* com15301 were grown in the presence of suberin-enriched potato periderm, production of vanillic acid was observed only in the mutant culture medium (Fig. 3.7. A). The accumulation of vanillic acid from the suberin-enriched potato periderm rapidly reached a plateau (Fig. 3.7. A). Accumulation of vanillate may interfere with ferulic acid degradation as suggested by the fact that strain ΔSCAB_15301 was less efficiently catabolizing ferulic acid when grown in the presence of both *trans*-ferulic and vanillic acids (Fig. 3.7. C) than with ferulic acid alone (Fig. 3.7. B).

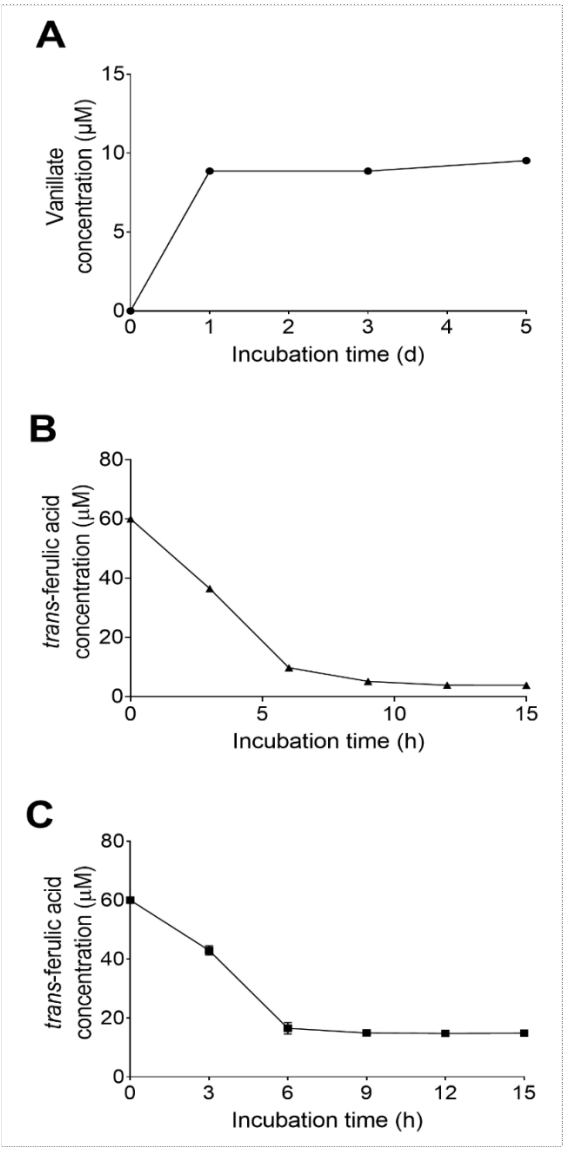

Figure 3.7. (A) Vanillate accumulation from suberin-enriched potato periderm in ΔSCAB_15301 (no accumulation was detected with the wild-type or the complemented strain). (B) Utilization of *trans*-ferulic acid in the ΔSCAB_15301 when grown in the presence of *trans*-ferulic acid. (C) Utilization of *trans*-ferulic acid in the ΔSCAB_15301 when grown in the presence of both *trans*-ferulic acid and vanillate. Data shown are the mean of three biological replicates (± SD).

Discussion

Beaulieu et al. (2016) previously demonstrated the ability of *S. scabies* to degrade the aliphatic moiety of suberin, but there has been no report about the degradation of the aromatic part of suberin by this pathogen. Nevertheless, a previous proteomic analysis revealed the presence of proteins predicted to play a role in the degradation of aromatic compounds when *S. scabies* was grown in the presence of suberin (Komeil et al., 2014). This study aimed to demonstrate the ability of *S. scabies* strains to utilize *trans*-ferulic and *p*-coumaric acids which are the main constituents of the suberin aromatic moiety.

In this study, *S. scabies* strains EF-35 and 87.22 were grown in a control medium supplemented with *trans*-ferulic and *p*-coumaric acids. Results revealed the ability of the two strains to efficiently degrade both substrates. The ability to degrade these cinnamic acids is shared by other streptomycetes such as *Streptomyces setonii* (Sutherland et al., 1983), *Streptomyces* sp. strain V-1 (Yang et al., 2013), *Streptomyces sannanensis* (Ghosh et al., 2007), *Streptomyces canus* GLY-P2 (Wu et al., 2019), *Streptomyces coelicolor* and *Streptomyces viridosporus* (Davis and Sello, 2010).

Sutherland et al. (1983) reported the ability of *Streptomyces setonii* to catabolize ferulic acid into vanillin, vanillic acid, and protocatechuic acid. The proteomic study of *S. scabies* 87.22 in the presence of ferulic acid allowed the identification of proteins that could also be involved in the conversion of ferulic acid into vanillate (a putative feruloyl-CoA hydratase and feruloyl-CoA synthetase) and further degradation of vanillate into protocatechuate (a putative vanillate monooxygenase, iron-sulfur oxidoreductase beta subunit and IclR-family transcriptional regulator). In contrast,

Streptomyces sannanensis was shown to bioconvert ferulic acid into vanillate without further degradation into protocatechuic acid, possibly because of the absence of vanillic acid demethylase and vanillic acid decarboxylase activities in this bacterium (Ghosh et al., 2007).

SCAB_15591 and SCAB_15601 genes, encoding putative feruloyl-CoA hydratase and feruloyl-CoA synthetase, respectively, which convert *trans*-ferulic acid into vanillate, were highly expressed in the presence of both ferulic and coumaric acids. This could be attributed to their broad substrate specificity. Masai et al. (2002) identified those two genes in *Sphingomonas paucimobilis* SYK-6 and showed that these enzymes exhibited broad substrate specificity as they were able to degrade *p*-coumaric acid, caffeic acid, and sinapinic acid. On the other hand, three genes were identified in *S. scabies* 87.22 to be potentially involved in the catabolism of vanillate (SCAB_15301, SCAB_15321 and SCAB_15331). The fact that those three genes were induced by ferulate suggests that they are involved in the conversion of vanillate into protocatechuic acid. These genes were found to be only induced in the presence of ferulic acid but not coumaric acid, suggesting their substrate specificity.

The predicted function of SCAB_15301 (vanillate monooxygenase) was confirmed by the fact that the mutant degraded *trans*-ferulic and accumulated vanillate as a degradation product. By contrast, in the wild-type strain, no accumulation of vanillic acid was detected when grown in the presence of *trans*-ferulic acid. Absence of ferulic acid degradation products suggests further metabolism of ferulate in *S. scabies* 87.22.

Previous work showed that when *p*-coumaric acid was used as an initial growth substrate for *S. setonii*, it was catabolized into *p*-hydroxybenzaldehyde, *p*-

hydroxybenzoic acid and protocatechuic acid (Sutherland et al., 1983). SCAB_2141 gene showed sequence homology with 2,3-dihydroxy-2,3-dihydro-phenylpropionate dehydrogenase, suggesting that this gene could be responsible for the conversion of *p*-coumaric acid into *p*-hydroxybenzoic acid. The fact that this gene was induced by coumaric acid but not by ferulic acid also supports this metabolic function.

Some proteins found in the proteome of *S. scabies* 87.22 in presence of ferulic acid were predicted to be involved in the catabolism of protocatechuate through the β-ketoadipate pathway (protocatechuate 3,4-dioxygenase beta subunit, 3-oxoadipate CoA-transferase subunit and 3-oxoadipate CoA-transferase subunit A). The β-ketoadipate pathway is a chromosomally encoded pathway that is widely distributed in soil bacteria and fungi (Harwood and Parales, 1996). This highly conserved pathway has two branches; one of them converts protocatechuate, derived from phenolic compounds such as ferulic and coumaric acids, to β- ketoadipate. The gene cluster for protocatechuic acid catabolism was characterized in *Streptomyces* sp. strain 2065 (Iwagami et al., 2000). In this strain, a protocatechuate 3,4-dioxygenase was purified and seven genes were identified to be involved in the degradation of protocatechuate via the β- ketoadipate pathway (Iwagami et al., 2000).

In several studies, it was reported that the expression of β-ketoadipate pathway genes was induced by aromatic compounds or by β-ketoadipate (Harwood and Parales, 1996). The high expression of genes involved in the β-ketoadipate pathway in the presence of both *trans*-ferulic and *p*-coumaric acids in *S. scabies* supports the hypothesis that these two phenolic compounds share a common degradation pathway (β-ketoadipate pathway). In ΔSCAB_15301, the gene SCAB_13631, which encodes the first key enzyme in the β-ketoadipate pathway, was not induced in the presence of ferulic acid, suggesting that neither ferulic nor vanillic acid was the real inducer of

the β-ketoadipate pathway. However, SCAB_13631 was induced in the presence of protocatechuate in both the mutant and the wild-type strains, suggesting that protocatechuate or its degradation products are the real inducers of the pathway. These results are in accordance with Davis and Sello (2010) who showed that both *S. coelicolor* and *S. viridosporus* contained the *pca* structural genes. These *pca* genes encode the enzymes of the protocatechuate branch of the β-ketoadipate pathway and were shown to be induced by protocatechuate and by *p*-hydroxybenzoate.

Most of the genes identified as being involved in degradation of aromatic compounds showed high expression in *S. scabies* 87.22 in the presence of potato suberin, which could be attributed to the hydroxycinnamates content of suberin (Bernards and Lewis, 1998; Bernards et al., 1995). However, their expression in the presence of suberin was much lower than in the presence of ferulic and coumaric acids. This could be due to the fact that suberin degradation is a slow process (Hamer et al., 2012; Beaulieu et al., 2016). Vanillic acid accumulated when ΔSCAB_15301 was grown in the presence of suberin, suggesting that *S. scabies* 87.22 can retrieve ferulic acid from potato periderm. Similarly, previous work revealed the ability of another actinobacterium, *S. cinnamoneus*, to release ferulic acid from biomass (Uraji et al., 2018).

Phenolic compounds were shown to induce stress in bacteria (Rivas-Sendra et al., 2011). *p*-coumaric acid can disturb protein structure, affect the properties of the cell membrane and interfere with DNA replication of *Lactobacillus casei* BL23 (Rivas-Sendra et al., 2011). The proteome analysis of *S. scabies* 87.22 in the presence of ferulic acid revealed the production of stress proteins, especially protein involved in acid stress tolerance. Among these proteins is a uracil-DNA glycosylase, which has been reported to be involved in DNA repair, mutation prevention and tolerance to acidified nitrite in G+C-rich bacteria (Venkatesh et al., 2003). An aspartate ammonia-

lyase (AspA) was also detected. This protein was shown to increase the acid survival in *Yersinia pseudotuberculosis* by producing ammonia (Hu et al., 2010). The α-acetolactate synthase (ALS) may contribute to *S. scabies* pH homeostasis following the addition of ferulic acid as an α-acetolactate synthase played this function in *Lactococcus lactis* under acid stress conditions (Zuljan et al., 2014). The most abundant protein found in the presence of ferulic acid only is a putative betaine aldehyde dehydrogenase. Since betaine is known to be an osmolyte produced in response to stress, especially to osmotic stress (Park et al., 1995), the induction of betaine aldehyde dehydrogenase in the presence of ferulic acid may represent a response to general stress. However, streptomycetes have be shown to produce several NADPH/NADH-producing enzymes in presence of recalcitrant compounds that could help to offset the low level of energy obtained from their catabolism (Sineli et al., 2018). These findings reveal that *S. scabies* 87.22 has undergone various adaptive physiological mechanisms in response to ferulic acid.

Streptomyces scabies also produced proteins involved in lipid metabolism after the addition of ferulic acid to its growth medium. In potato periderm, ferulate esters are important structural elements but represent only a small fraction of suberin constituents which is mainly composed of fatty acids (Beaulieu et al., 2016). Therefore, ferulic acid might act as a signal to produce enzymes such as those of the fatty acids oxidation complex. This protein complex which breaks down CoA activated derivatives of fatty acids into acetyl-CoA by the β-oxidation pathway (Menendez-Bravo et al., 2017) might be important for the degradation of the aliphatic constituents of suberin. Interestingly, the second most abundant protein induced by the addition of ferulic acid, SCAB_3954, is encoded by a gene of unknown function that is located in a gene cluster dedicated to Coenzyme A biosynthesis. Such relationship between fatty acid metabolism and catabolism of aromatic compounds has also been suggested in another actinobacterium, *Rhodococcus* strain BUBNP1. A transcriptomics study

revealed that strain BUBNP1 degrades concurrently 4-nitrophenol and fatty acids (Sengupta et al., 2019).

Our study indicates that *S. scabies* can utilize hydroxycinnamates, contained in suberin, as carbon and energy sources via the β-ketoadipate pathway. This may be advantageous in environments where carbon sources are limited. In the last step of the β-ketoadipate pathway, once β-ketoadipyl-CoA is formed, it is converted to succinyl-CoA and acetyl-CoA. The latter two compounds are further metabolized via Krebs cycle, yielding energy. This could offer not only an ecological advantage to *S. scabies* as a saprophyte but also as a pathogen. Further research is required to elucidate the importance of degradation of aromatic compounds via β-ketoadipate in *S. scabies* environment, as it was reported before that the catabolism of aromatic compounds through β-ketoadipate pathway is necessary for the pathogenicity of other plant pathogens such as *Fusarium oxysporum* (Michielse et al., 2012).

Author contributions statement

MK and CB designed the experiments. MK performed the lab work and analyzed the data with the help of SL who generated the mutant strain. MK and CB wrote the manuscript, with the critical review of CB, NB and SL. CB supervised the project. All authors approved the final version of the manuscript.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Table S1. Supplementary Table. Proteins produced by *Streptomyces scabies* 87.22 during growth in CM supplemented or not with *trans*-ferulic acid.

			NSpC	
Uniprot accession number	**Corresponding gene in *S. scabies* 87.22**	**Putative protein function**	**Control medium**	**Control medium with ferulic acid**
Carbohydrate transport and metabolism				
C9YW88	SCAB_37051	Beta-xylanase	0.45	0.40
C9Z433	SCAB_42161	Fructose 1,6-bisphosphate aldolase	0.28	0.49
C9ZGR4	SCAB_34111	Phosphoenolpyruvate carboxykinase	0.16	0.22
C9Z6V6	SCAB_27951	Glycogen phosphorylase	0.07	0.04
C9Z9C4	SCAB_76231	Mannose-1-phosphate guanyltransferase	0.12	0.12
C9YY64	SCAB_69721	Triosephosphate isomerase	0.06	0.09
C9ZF65	SCAB_33521	Fructose-1,6-bisphosphatase	0.34	0.32
C9ZAM6	SCAB_46011	1L-myo-inositol-1-phosphate synthase	0.17	0.24
C9ZDV4	SCAB_63781	Alpha-L-arabinofuranosidase	0.05	0.06
C9YY84	SCAB_69921	Transketolase	0.11	0.15
C9YTW6	SCAB_67551	Glucokinase	0.14	0.14

C9YUT7	SCAB_35801	Succinate--CoA ligase	0.17	0.15
C9Z5N4	SCAB_43191	Hydrolase	0.33	0.37
C9YU13	SCAB_81861	Pyruvate carboxylase	0.02	0.07
C9YY62	SCAB_69701	Glyceraldehyde-3-phosphate dehydrogenase	0.40	0.33
C9YUT6	SCAB_35791	Succinyl-CoA ligase	0.15	0.20
C9YT49	SCAB_19421	Oxidoreductase	0.08	0.07
C9ZH18	SCAB_49941	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	0.23	0.20
C9Z545	SCAB_11811	6-phosphogluconate dehydrogenase, decarboxylating	0.14	0.13
C9YY63	SCAB_69711	Phosphoglycerate kinase	0.13	0.12
C9ZH59	SCAB_50381	Trehalose-phosphate synthase	0.12	0.15
C9Z4A0	SCAB_58271	Sugar hydrolase	0.17	0.15
C9YY82	SCAB_69901	Glucose-6-phosphate 1-dehydrogenase	0.17	0.13
C9ZBJ5	SCAB_15481	Alpha-mannosidase	0.08	0.09
C9Z109	SCAB_55931	Malate dehydrogenase	0.24	0.21
C9Z737	SCAB_43661	Secreted protein	0.20	0.19
C9Z5R8	SCAB_58791	Citrate synthase	0.36	0.30
C9YY67	SCAB_69751	Glucose-6-phosphate isomerase (GPI)	0.14	0.10
C9ZAT6	SCAB_62141	Pyruvate phosphate dikinase	0.22	0.26

C9YY83	SCAB_69911	Transaldolase	0.49	0.48
C9ZFY5	SCAB_79861	Xylose isomerase	0.43	0.36
C9YY37	SCAB_54441	Enolase	0.69	0.68
Stress mechanism				
C9Z7C3	SCAB_59681	Secreted peptidase	0.08	0.09
C9ZE08	SCAB_64341	Stress protein	1.03	0.78
C9Z7C8	SCAB_59731	Superoxide dismutase	0.48	0.48
C9ZEQ9	SCAB_17101	ATP/GTP binding protein	0.25	0.18
C9ZAA4	SCAB_29931	Nickel superoxide dismutase	0.57	0.57
C9Z721	SCAB_43491	Uncharacterized protein	0.40	0.33
C9YYC2	SCAB_70311	Stress-induced protein	0.58	0.61
C9Z785	SCAB_44161	Hydrolase	0.33	0.23
C9ZAL8	SCAB_45931	Thioredoxin reductase	0.40	0.35
C9Z1W0	SCAB_9531	Catalase-peroxidase	0.37	0.34
C9YWU0	SCAB_69441	Stress-induced protein	0.79	0.52
C9ZE07	SCAB_64331	Stress protein	2.33	1.58
C9ZH47	SCAB_50261	Stress protein	3.00	2.00
C9ZHS9	SCAB_81661	Tellurium resistance protein	2.58	1.98
Lipid metabolism				

C9YTK3	SCAB_51091	Secreted peptidase	0.61	0.44
C9Z707	SCAB_28481	Acetyl-coA C-acetyltransferase FadA	0.52	0.54
C9ZAG3	SCAB_45341	3-hydroxyacyl-CoA dehydrogenase	0.16	0.13
C9ZGV4	SCAB_34521	Enoyl-CoA hydratase	0.36	0.17
C9Z5Z2	SCAB_74351	Secreted glycerophosphoryl diester phosphodiesterase	0.20	0.17
C9YZS0	SCAB_70981	Enoyl-CoA hydratase/isomerase	0.09	0.09
C9Z6U1	SCAB_27801	Enoyl-coA hydratase/isomerase family protein	0.24	0.13
C9ZDY7	SCAB_64131	3-oxoacyl-[acyl-carrier-protein] synthase 2	0.07	0.07
C9Z0I2	SCAB_24201	Uncharacterized protein	0.31	0.38
C9Z9F5	SCAB_76541	3-oxoacyl-[acyl-carrier protein] reductase	0.15	0.19
C9Z8D6	SCAB_28641	3-hydroxybutyryl-coA dehydrogenase	0.23	0.22
C9Z1F6	SCAB_72321	Secreted lipase	0.10	0.28
C9Z1E2	SCAB_72171	Acyl-CoA dehydrogenase	0.12	0.09
C9YYH5	SCAB_83811	Dihydroxyacetone kinase component	0.10	0.09
C9YZG6	SCAB_54931	Acyl-CoA dehydrogenase	0.10	0.12
C9Z4I9	SCAB_74071	3-hydroxyacyl-CoA dehydrogenase	0.15	0.20
C9ZDZ0	SCAB_64161	Malonyl CoA:acyl carrier protein malonyltransferase	0.39	0.31
C9YX75	SCAB_6631	Acyl-CoA thiolase	0.06	0.20
C9Z2I3	SCAB_41241	Acetyl-coenzyme A synthetase	0.13	0.09

C9YZV5	SCAB_71331	Enoyl-[acyl-carrier-protein] reductase [NADH]	0.31	0.28
C9Z6Y6	SCAB_28271	Cholesterol esterase	0.70	0.63
C9YY49	SCAB_54571	Acetyl-CoA C-acyltransferase	0.34	0.44
Transcriptional, translational, ribosomal structure and biogenesis				
C9YWA2	SCAB_37191	DNA-directed RNA polymerase subunit beta	0.37	0.37
C9Z240	SCAB_25251	Polyribonucleotide nucleotidyltransferase	0.59	0.53
C9YW51	SCAB_36671	DNA-directed RNA polymerase subunit alpha	0.97	1.07
C9YW66	SCAB_36821	50S ribosomal protein L5	1.38	1.50
C9YW75	SCAB_36911	50S ribosomal protein L2	0.83	0.78
C9YW64	SCAB_36801	30S ribosomal protein S8	1.96	1.96
C9YW72	SCAB_36881	30S ribosomal protein S3	0.82	0.78
C9YW78	SCAB_36941	50S ribosomal protein L3	0.76	0.80
C9ZE09	SCAB_64351	Integral membrane protein	0.21	0.20
C9Z7K6	SCAB_75261	Uncharacterized protein	1.38	1.00
C9Z241	SCAB_25261	30S ribosomal protein S15	1.32	1.23
C9Z3N7	SCAB_25991	Ribosome-recycling factor	0.57	0.38
C9Z0H0	SCAB_24081	Two-component response regulator	0.38	0.38
C9YVQ3	SCAB_6061	Two-component system response regulator	0.38	0.28
C9Z4I0	SCAB_73981	50S ribosomal protein L20	0.71	0.71

C9ZAK6	SCAB_45811	Beta sliding clamp	0.26	0.24
C9ZAN3	SCAB_46101	30S ribosomal protein S6	1.00	0.82
C9YW59	SCAB_36751	50S ribosomal protein L15	0.63	0.59
C9YW70	SCAB_36861	50S ribosomal protein L29	1.38	1.06
C9YW74	SCAB_36901	30S ribosomal protein S19	0.86	0.91
C9YW53	SCAB_36691	30S ribosomal protein S13	0.61	0.71
C9Z629	SCAB_74751	Threonine--tRNA ligase	0.09	0.11
C9Z316	SCAB_73401	Uncharacterized protein	0.29	0.30
C9YW77	SCAB_36931	50S ribosomal protein L4	0.48	0.37
C9YW67	SCAB_36831	50S ribosomal protein L24	1.00	0.96
C9YW79	SCAB_36951	30S ribosomal protein S10	0.83	1.13
C9YW62	SCAB_36781	50S ribosomal protein L18	0.54	0.21
C9YW47	SCAB_36631	50S ribosomal protein L13	0.84	1.13
C9YW76	SCAB_36921	50S ribosomal protein L23	0.83	0.53
C9YW95	SCAB_37121	30S ribosomal protein S12	0.75	0.71
C9YZB2	SCAB_38941	Lysine--tRNA ligase	0.15	0.10
C9Z2I8	SCAB_56731	Ribonuclease PH (RNase PH)	0.25	0.37
C9Z3Q3	SCAB_26151	50S ribosomal protein L19	0.92	0.88
C9YW50	SCAB_36661	50S ribosomal protein L17	0.94	0.81

C9YW93	SCAB_37101	Elongation factor G	0.20	0.26
C9YZB3	SCAB_38961	Arginine--tRNA ligase	0.10	0.14
C9Z4S3	SCAB_87641	Ribonuclease J	0.13	0.15
C9ZAN4	SCAB_46111	Single-stranded DNA-binding protein	0.55	0.34
C9YWV2	SCAB_69561	UvrABC system protein A	0.04	0.04
C9Z3N9	SCAB_26011	Elongation factor Ts	0.35	0.45
C9YW63	SCAB_36791	50S ribosomal protein L6	0.97	1.13
C9Z2D8	SCAB_40781	DNA topoisomerase 1	0.17	0.22
C9YW52	SCAB_36681	30S ribosomal protein S11	0.57	0.57
C9YZ53	SCAB_38351	AsnC-family transcriptional regulator	0.38	0.28
C9YW61	SCAB_36771	30S ribosomal protein S5	0.95	1.00
C9YWA6	SCAB_37231	50S ribosomal protein L1	0.15	0.27
C9YWA4	SCAB_37211	50S ribosomal protein L10	0.29	0.42
C9Z8T7	SCAB_44991	Aspartate--tRNA ligase	0.09	0.11
C9ZAK2	SCAB_45761	DNA gyrase subunit B	0.07	0.10
C9YW73	SCAB_36891	50S ribosomal protein L22	0.73	0.38
C9YWP5	SCAB_68981	DNA polymerase I	0.05	0.06
C9Z2E6	SCAB_40861	Anti-sigma factor antagonist	0.42	0.29
C9Z7H2	SCAB_60151	50S ribosomal protein L21	0.58	0.46

C9Z7J7	SCAB_75171	BldD regulator of morphogenesis and anitbiotic production	0.28	0.33
C9Z7Q6	SCAB_75801	AsnC-family transcriptional regulatory protein	0.22	0.25
C9YV68	SCAB_52291	Chitinase-promoter-binding protein	0.17	0.12
C9Z0W7	SCAB_40011	50S ribosomal protein L28	0.50	0.38
C9YWA3	SCAB_37201	50S ribosomal protein L7/L12	0.23	0.31
C9Z257	SCAB_25421	Proline--tRNA ligase	0.06	0.06
C9ZAN5	SCAB_46121	30S ribosomal protein S18	0.39	0.44
C9YW68	SCAB_36841	50S ribosomal protein L14	1.58	1.65
C9YZR9	SCAB_70971	Uncharacterized protein	0.75	0.56
C9YV23	SCAB_51821	DNA-binding protein	0.17	0.11
C9ZAN6	SCAB_46131	50S ribosomal protein L9	0.19	0.31
C9YY11	SCAB_54151	50S ribosomal protein L25	0.21	0.21
C9YW71	SCAB_36871	50S ribosomal protein L16	0.47	0.38
C9Z8G5	SCAB_28931	Transcription termination factor Rho	0.04	0.05
C9Z978	SCAB_61451	30s ribosomal protein S20	0.44	0.33
C9Z3V0	SCAB_41311	Cyclic-nucleotide-binding protein	0.30	0.20
C9YW46	SCAB_36621	30S ribosomal protein S9	1.34	1.11
C9YW69	SCAB_36851	30S ribosomal protein S17	2.09	2.14
C9Z7H3	SCAB_60161	50S ribosomal protein L27	0.39	0.33

C9ZAK1	SCAB_45751	DNA gyrase subunit A	0.26	0.29
C9Z459	SCAB_57831	A-factor-responsive transcriptional activator	0.08	0.08
C9YWA8	SCAB_37251	Transcription termination/antitermination protein NusG	0.20	0.16
C9ZAC1	SCAB_30101	Sigma factor	0.06	0.06
C9Z2B7	SCAB_40551	Cysteine--tRNA ligase	0.15	0.11
C9Z233	SCAB_25181	Lactamase-b family hydrolase	0.48	0.43
C9Z3V4	SCAB_41351	Endoribonuclease	0.31	0.34
C9YZC9	SCAB_39121	AdsA-like sigma factor	0.23	0.13
C9Z656	SCAB_75031	30S ribosomal protein S4	1.19	1.23
C9YWQ2	SCAB_69061	30S ribosomal protein S1	0.55	0.50
C9Z0J8	SCAB_24391	DNA topoisomerase (ATP-hydrolyzing)	0.04	0.05
C9YW94	SCAB_37111	30S ribosomal protein S7	1.76	1.59
C9Z0M0	SCAB_24631	Vitamin B12-dependent ribonucleotide reductase	0.05	0.07
C9Z3P0	SCAB_26021	30S ribosomal protein S2	1.24	1.22
C9YW92	SCAB_37091	Elongation factor Tu	0.68	0.97
C9YWA1	SCAB_37181	DNA-directed RNA polymerase subunit beta	0.57	0.54
Nucleotide transport and metabolism				
C9YWT5	SCAB_69391	Pseudouridine-5'-phosphate glycosidase	0.31	0.36
C9ZB78	SCAB_78051	Adenylosuccinate lyase	0.21	0.13

C9YWF6	SCAB_53071	Purine nucleoside phosphorylase	0.16	0.16
C9YZE5	SCAB_54711	Peptidase	0.07	0.08
C9YUT1	SCAB_35741	Bifunctional purine biosynthesis protein PurH	0.05	0.07
C9ZGX4	SCAB_49491	Secreted 5'-nucleotidase	0.17	0.13
C9ZDQ0	SCAB_47701	Phosphoribosylamine--glycine ligase	0.12	0.08
C9YZ58	SCAB_38401	3-octaprenyl-4-hydroxybenzoate carboxy-lyase	0.07	0.08
C9ZGW8	SCAB_34681	Purine nucleoside phosphorylase	0.26	0.24
C9Z7K4	SCAB_75241	Dihydroorotate dehydrogenase (quinone)	0.06	0.09
C9Z7F8	SCAB_60011	Nucleoside diphosphate kinase	1.00	0.83
C9Z5I3	SCAB_42681	dCTP deaminase	0.14	0.12
C9YVD1	SCAB_68051	Pyridoxal phosphate homeostasis protein	0.16	0.18
C9YVK8	SCAB_68841	5' nucleotidase	0.15	0.15
C9ZAW5	SCAB_62461	Deoxyguanosinetriphosphate triphosphohydrolase-like protein	0.09	0.09
C9YUX6	SCAB_36211	Inosine-5'-monophosphate dehydrogenase	0.36	0.45
C9Z407	SCAB_41891	Adenylosuccinate synthetase	0.30	0.29
C9Z995	SCAB_61621	Hit-family protein	0.38	0.38
C9YY13	SCAB_54171	Bifunctional protein GlmU	0.12	0.18

Cell wall/membrane/envelope biogenesis

C9YV70	SCAB_52311	D-alanyl-D-alanine carboxypeptidase	0.07	0.07
C9Z111	SCAB_55951	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	0.29	0.40
C9Z836	SCAB_13061	Nucleotide sugar-1-phosphate transferase	0.13	0.11
C9Z2W0	SCAB_72801	Secreted protein	0.14	0.17
C9Z8V2	SCAB_45141	D-alanyl-D-alanine carboxypeptidase	0.68	0.57
C9YT92	SCAB_34981	Lipoprotein	1.53	1.09
C9Z994	SCAB_61611	Protease	0.33	0.35
Coenzyme transport and metabolism				
C9Z3L8	SCAB_25801	Adenosine/AMP deaminase	0.15	0.11
C9Z7P0	SCAB_75631	6,7-dimethyl-8-ribityllumazine synthase	0.71	0.71
C9YYP1	SCAB_7581	Hydroxymethylbilane synthase	0.15	0.13
C9Z8E0	SCAB_28681	Cobalamin adenosyltransferase	0.17	0.14
C9YZQ8	SCAB_70851	Cob(I)yrinic acid a,c-diamide adenosyltransferase	0.14	0.14
C9YUS8	SCAB_35711	Bifunctional protein FolD	0.27	0.17
C9Z3T0	SCAB_26421	Phosphopantetheine adenylyltransferase	0.19	0.22
C9Z638	SCAB_74841	Pyridoxal 5'-phosphate synthase subunit PdxS	0.93	0.74
C9Z1Y4	SCAB_9771	Probable cobalamin biosynthesis protein cobN	0.51	0.47
C9ZBZ9	SCAB_46341	Phosphomethylpyrimidine synthase	0.07	0.07
C9Z3M4	SCAB_25861	Aminotransferase	0.29	0.29

Amino acid transport and metabolism

C9YTK4	SCAB_51101	Phosphoserine aminotransferase	0.56	0.55
C9ZHG5	SCAB_66881	Glutamine synthetase	0.49	0.56
C9Z204	SCAB_24891	Glutamate uptake system binding subunit	1.50	1.34
C9Z7L0	SCAB_75311	S-adenosylmethionine synthase	0.36	0.35
C9ZAW6	SCAB_62471	Secreted aminopeptidase	0.30	0.25
C9Z7C5	SCAB_59701	Aminopeptidase	0.13	0.19
C9Z234	SCAB_25191	4-hydroxy-tetrahydrodipicolinate synthase	0.56	0.48
C9ZGG7	SCAB_18081	Gamma-glutamyltranspeptidase	0.13	0.12
C9Z7H9	SCAB_60221	Gamma-glutamyl phosphate reductase	0.26	0.27
C9ZGT9	SCAB_34371	Cystathionine gamma-synthase	0.30	0.27
C9Z9D4	SCAB_76331	Glycine dehydrogenase	0.20	0.23
C9Z5A0	SCAB_27061	Ketol-acid reductoisomerase	0.47	0.53
C9Z1B0	SCAB_71831	Alanine dehydrogenase	0.20	0.26
C9Z8G7	SCAB_28951	Threonine synthase	0.16	0.34
C9Z238	SCAB_25231	4-hydroxy-tetrahydrodipicolinate reductase	0.42	0.27
C9ZC37	SCAB_46731	Xaa-Pro aminopeptidase	0.45	0.35
C9Z443	SCAB_42271	Aspartate aminotransferase	0.18	0.16
C9Z483	SCAB_58101	Guanidinobutyrase	0.29	0.20

C9YTR8	SCAB_67071	Probable cytosol aminopeptidase	0.63	0.52
C9Z593	SCAB_26991	Aldehyde dehydrogenase	0.07	0.10
C9Z2Y0	SCAB_73011	Peptidase	0.06	0.08
C9Z4K3	SCAB_74211	Arginine biosynthesis bifunctional protein ArgJ	0.08	0.10
C9Z281	SCAB_25691	4-aminobutyrate aminotransferase	0.63	0.53
C9ZCM3	SCAB_78471	Ornithine aminotransferase	0.16	0.14
C9YY98	SCAB_70071	Cysteine desulfurase	0.17	0.14
C9ZC04	SCAB_46391	Phenylalanine aminotransferase	0.21	0.21
C9YYA5	SCAB_70141	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase	0.13	0.18
C9Z2X8	SCAB_72981	Secreted metallopeptidase	0.08	0.14
C9ZGG2	SCAB_18021	Uncharacterized protein	0.08	0.04
C9YZJ1	SCAB_55181	Adenosylhomocysteinase	0.16	0.21
C9Z6N6	SCAB_12671	2-amino-3-ketobutyrate coenzyme A ligase	0.12	0.10
C9Z4K2	SCAB_74201	N-acetyl-gamma-glutamyl-phosphate reductase	0.17	0.16
C9YYX2	SCAB_23141	Ornithine carbamoyltransferase	0.07	0.08
C9YVG2	SCAB_68371	Imidazoleglycerol-phosphate dehydratase	0.11	0.14
C9YYX3	SCAB_23151	Arginine deiminase	0.11	0.10
C9ZBY2	SCAB_31381	Dipeptidyl-peptidase IV	0.07	0.05

C9Z4G8	SCAB_73851	Glutamine synthetase	0.07	0.06
C9Z7B6	SCAB_59611	Aminopeptidase N	0.10	0.11
C9YVH4	SCAB_68491	Indole-3-glycerol phosphate synthase	0.16	0.11
C9Z8U5	SCAB_45071	Probable M18 family aminopeptidase 2	0.21	0.22
C9Z7P2	SCAB_75651	ATP phosphoribosyltransferase	0.19	0.19
C9ZAQ8	SCAB_61851	2-isopropylmalate synthase	0.04	0.06
C9ZHC2	SCAB_66451	Glutamine-dependent NAD(+) synthetase	0.19	0.17
C9ZGV2	SCAB_34501	Histidine ammonia-lyase	0.46	0.41
C9YWB0	SCAB_37271	Aspartate aminotransferase	0.32	0.31
C9YZE4	SCAB_54701	Urocanate hydratase	0.75	0.55
C9YWP0	SCAB_68931	Secreted binding protein	0.79	1.01
Transport, secretion and efflux				
C9Z5D4	SCAB_27411	Secreted oligopeptide-binding transport system protein	1.12	1.26
C9ZFJ5	SCAB_49311	Phosphate-binding protein PstS	0.83	0.82
C9YTX8	SCAB_67681	Bacterioferritin	0.84	0.79
C9ZA98	SCAB_29881	Binding protein	0.38	0.36
C9Z0C3	SCAB_8741	Solute-binding lipoprotein	0.32	0.21
C9Z0P2	SCAB_24881	Glutamate uptake system ATP-binding subunit	0.45	0.29
C9YWN6	SCAB_68891	ABC transporter ATP-binding subunit	0.29	0.35

C9YY97	SCAB_70061	ABC transporter ATP-binding subunit	0.14	0.25
C9YUK3	SCAB_19841	Lipoprotein	0.15	0.13
C9Z205	SCAB_24901	Glutamate uptake system	0.27	0.15
C9ZH25	SCAB_50031	Phosphate-specific transport system accessory protein PhoU	0.16	0.24
C9Z0I9	SCAB_24291	DNA topoisomerase	0.11	0.08
C9ZAS0	SCAB_61971	Binding-protein-dependent metal transporter ATP-binding subunit	0.24	0.41
C9YZP9	SCAB_70761	Secreted solute-binding protein	0.30	0.27
C9Z5D2	SCAB_27391	Oligopeptide ABC transporter component	0.28	0.24
C9Z3R1	SCAB_26231	Signal recognition particle protein	0.10	0.08
C9YZK9	SCAB_55371	Protein translocase subunit SecA	0.05	0.04
C9YX53	SCAB_83561	L-asparagine permease	0.09	0.09
C9Z206	SCAB_24911	Glutamate uptake system	0.09	0.09
C9YUG0	SCAB_5331	ATP-binding component of ABC transporter	0.09	0.22
C9YWN9	SCAB_68921	ABC transporter integral membrane subunit	0.18	0.11
C9YTT3	SCAB_67221	Integral membrane efflux protein	0.05	0.06
C9Z7A7	SCAB_59521	Mechanosensitive ion channel	0.05	0.08
C9ZA99	SCAB_29891	Binding-protein-dependent transporter	0.25	0.28

C9YWN8	SCAB_68911	ABC transporter integral membrane subunit	0.08	0.05
C9Z8C6	SCAB_28551	ABC transporter ATP-binding subunit	0.09	0.09
C9ZBZ2	SCAB_31501	Peptide ABC transporter	0.11	0.10
C9Z307	SCAB_73301	Glycerol uptake facilitator protein	0.22	0.13
C9YW00	SCAB_21251	RNA helicase	0.04	0.03
C9Z5D1	SCAB_27381	Oligopeptide ABC transporter	0.15	0.16
C9ZD96	SCAB_31521	BldKC-like oligopeptide ABC transporter subunit	0.08	0.11
C9ZE01	SCAB_64271	Efflux transporter	0.04	0.05
C9ZD98	SCAB_31541	BldKA-like ABC transport system integral membrane protein	0.13	0.05
C9YT14	SCAB_19051	Probable solute-binding lipoprotein	0.44	0.37
C9Z646	SCAB_74921	Protein-export membrane protein SecF	0.08	0.09
C9ZD95	SCAB_31511	BldKD oligopeptide ABC transporter subunit	0.36	0.24
C9Z000	SCAB_84391	Cobalt transport protein CbiN	0.23	0.20
C9ZD97	SCAB_31531	BldKB-like transport system extracellular solute-binding protein	0.39	0.42
C9Z2T0	SCAB_57661	Secreted solute-binding protein	0.66	0.56
C9YUG2	SCAB_5351	Substrate-binding component of ABC transporter	0.66	0.73
C9YVX8	SCAB_21021	Secreted solute-binding protein	0.79	0.87

Energy production and conversion

C9YTR7	SCAB_67061	Dihydrolipoyl dehydrogenase	2.74	2.35
C9ZGW6	SCAB_34651	Oxidoreductase	0.62	0.66
C9YTC3	SCAB_35291	Succinate dehydrogenase flavoprotein subunit	0.45	0.50
C9Z8F0	SCAB_28781	ATP synthase subunit alpha	1.70	1.64
C9Z8F1	SCAB_28791	ATP synthase subunit delta	0.84	0.71
C9YTU0	SCAB_67291	Cytochrome c oxidase subunit II	0.66	0.50
C9YTR4	SCAB_67031	Pyruvate dehydrogenase E1 component	0.11	0.16
C9Z6Y2	SCAB_28231	Acetate kinase	0.28	0.19
C9YWX6	SCAB_82781	Hydrogenase	0.31	0.41
C9ZA89	SCAB_29781	2-oxoglutarate dehydrogenase	0.03	0.08
C9Z0U9	SCAB_39831	Inorganic pyrophosphatase	0.37	0.39
C9Z476	SCAB_58021	Acetyl/propionyl CoA carboxylase alpha subunit	0.15	0.17
C9Z7Q3	SCAB_75771	Acyl-CoA dehydrogenase	0.29	0.18
C9YTC4	SCAB_35301	Succinate dehydrogenase iron-sulfur subunit	0.34	0.29
C9YTU1	SCAB_67301	Cytochrome c oxidase subunit I	0.13	0.14
C9Z8V4	SCAB_45161	E2 branched-chain alpha keto acid dehydrogenase system	0.26	0.25
C9YYR1	SCAB_7801	Oxidoreducatse	0.10	0.07
C9ZAG4	SCAB_45351	Aldehyde dehydrogenase	0.20	0.22

C9Z6J6	SCAB_12211	Isocitrate dehydrogenase	0.16	0.21
C9YXW4	SCAB_38111	Oxidoreductase	0.20	0.14
C9Z306	SCAB_73291	Glycerol kinase	0.27	0.16
C9YVY6	SCAB_21101	Aconitate hydratase	0.18	0.25
C9YWN7	SCAB_68901	ABC transporter ATP-binding subunit	0.41	0.36
C9Z8F2	SCAB_28801	ATP synthase subunit b	1.35	1.35
C9YXQ1	SCAB_37431	NADH dehydrogenase subunit NuoM2	0.05	0.06
C9Z8F4	SCAB_28821	ATP synthase subunit a	0.24	0.22
C9YZL4	SCAB_55421	NAD-glutamate dehydrogenase	0.02	0.04
C9YTC2	SCAB_35281	Succinate dehydrogenase membrane subunit	0.11	0.11
C9Z5T2	SCAB_58931	Methylmalonic acid semialdehyde dehydrogenase	0.10	0.09
C9ZEP5	SCAB_16961	Oxidoreductase	0.10	0.12
C9Z8V5	SCAB_45171	E1-beta branched-chain alpha-keto-acid dehydrogenase system	0.19	0.20
C9ZE04	SCAB_64301	Pyruvate dehydrogenase E1 component	0.04	0.05
C9ZA96	SCAB_29851	Alcohol dehydrogenase	0.10	0.09
C9YTU7	SCAB_67361	Ubiquinol-cytochrome c reductase iron-sulfur subunit	0.09	0.16
C9ZFY0	SCAB_79811	Aldehyde dehydrogenase	0.06	0.06
C9ZBX1	SCAB_31271	Ferredoxin	0.67	0.42

C9ZF68	SCAB_33551	Fumarate hydratase class I	0.07	0.05
C9Z8V6	SCAB_45181	E1-alpha branched-chain alpha keto acid dehydrogenase system	0.08	0.16
C9YTM7	SCAB_51341	Citrate synthase	0.15	0.14
C9YUX5	SCAB_36201	IMP dehydrogenase/ GMP reductase	0.51	0.64
C9YTR6	SCAB_67051	Dihydrolipoyllysine-residue succinyltransferase	0.38	0.38
C9Z8E8	SCAB_28761	ATP synthase subunit beta	1.54	1.77
C9YTG2	SCAB_35681	Malate dehydrogenase	1.90	1.59
C9Z8E9	SCAB_28771	ATP synthase gamma chain	1.15	1.18
General function prediction only				
C9ZH64	SCAB_50441	60 Chaperonin	0.64	0.86
C9ZD54	SCAB_16471	DNA-binding protein	1.00	0.96
C9Z5G9	SCAB_42541	Chaperone protein DnaK	0.37	0.36
C9Z0R6	SCAB_39491	Clp-family ATP-binding protease	0.25	0.23
C9Z0U4	SCAB_39781	ATP-dependent zinc metalloprotease FtsH	0.22	0.27
C9ZCL9	SCAB_78431	Secreted tripeptidylaminopeptidase	0.28	0.25
C9YZC0	SCAB_39031	Delta-aminolevulinic acid dehydratase	0.35	0.35
C9YVQ4	SCAB_6071	Histidine kinase	0.34	0.22
C9Z2T5	SCAB_72541	Homogentisate 1,2-dioxygenase	0.21	0.24

C9Z3Q0	SCAB_26121	Signal peptidase	0.33	0.34
C9Z5E5	SCAB_42291	Chaperone protein ClpB	0.12	0.10
C9YUY5	SCAB_36301	10 Chaperonin	0.91	0.91
C9Z7F0	SCAB_59941	ATP-dependent Clp protease proteolytic subunit	0.30	0.34
C9ZAJ2	SCAB_45651	Peptidyl-prolyl cis-trans isomerase	0.34	0.39
C9Z0V9	SCAB_39931	Aldehyde dehydrogenase	0.17	0.12
C9ZGZ0	SCAB_49661	Uncharacterized protein	0.65	0.46
C9YXV0	SCAB_37971	NuoM, NADH dehydrogenase subunit	0.13	0.13
C9Z4D5	SCAB_73521	Peptidyl-prolyl cis-trans isomerase	0.26	0.15
C9Z474	SCAB_58001	Acyl-CoA dehydrogenase	0.20	0.18
C9Z589	SCAB_26951	3-isopropylmalate dehydrogenase	0.17	0.18
C9Z9C8	SCAB_76271	Uncharacterized protein	0.24	0.29
C9Z0Y6	SCAB_55671	Serine/threonine protein kinase	0.08	0.07
C9YXC1	SCAB_7101	Alcohol dehydrogenase class III	0.15	0.09
C9YUI1	SCAB_5561	Integral membrane protein	0.22	0.24
C9Z8W9	SCAB_45311	E1-alpha branched-chain alpha keto acid dehydrogenase	0.15	0.20
C9Z7I2	SCAB_60251	Peptidase	0.18	0.21
C9Z1U3	SCAB_9361	Prolyl aminopeptidase	0.08	0.08
C9Z210	SCAB_24951	Protein RecA	0.11	0.28

C9Z1F7	SCAB_72331	Hydrolase	0.15	0.19
C9ZAH6	SCAB_45481	Secreted protein	0.16	0.18
C9Z475	SCAB_58011	Hydroxymethylglutaryl-CoA lyase	0.32	0.29
C9YZ81	SCAB_38631	Cytochrome assembly protein	0.08	0.11
C9YYG1	SCAB_83671	Esterase	0.07	0.10
C9YZK1	SCAB_55281	Two-component system response regulator	0.14	0.22
C9ZGF2	SCAB_17921	Oxidoreductase	0.15	0.19
C9Z110	SCAB_55941	DNA-binding protein HU1/hs1	0.45	0.65
C9ZAC0	SCAB_30091	Anti-sigma factor	0.20	0.20
C9Z421	SCAB_42041	Cytochrome P-450 hydroxylase	0.07	0.06
C9Z6A5	SCAB_88391	2-hydroxyhepta-2,4-diene-1,7-dioate isomerase	0.10	0.06
C9Z1Q3	SCAB_85931	Regulatory protein	0.09	0.06
C9ZAL7	SCAB_45921	Thioredoxin	0.21	0.17
C9ZAI3	SCAB_45561	Penicillin-binding kinase	0.04	0.07
C9Z7F1	SCAB_59951	ATP-dependent Clp protease proteolytic subunit	0.20	0.26
C9ZC20	SCAB_46561	Serine--tRNA ligase	0.05	0.09
C9Z477	SCAB_58031	Acetyl/propionyl CoA carboxylase, beta subunit	0.21	0.24
C9ZD20	SCAB_16121	Serine/threonine-protein kinase	0.06	0.05
C9YWG0	SCAB_53111	Secreted penicillin acylase	0.35	0.27

C9ZDR6	SCAB_47861	Phosphoribosylformylglycinamidine cyclo-ligase	0.50	0.46
C9YUY4	SCAB_36291	60 Chaperonin	0.51	0.50
Unknown function				
C9Z4C0	SCAB_58481		0.74	0.79
C9YVE9	SCAB_68241		0.41	0.47
C9Z3A4	SCAB_86841		1.21	1.32
C9YZX4	SCAB_71541		0.56	0.46
C9ZBU0	SCAB_30961		0.46	0.57
C9ZB66	SCAB_77921		0.22	0.22
C9ZBP7	USCAB_30501		0.11	0.14
C9ZH08	SCAB_49851		0.57	0.64
C9Z064	SCAB_85031		0.72	0.67
C9YWC0	SCAB_37371		0.72	0.58
C9YUE9	SCAB_5221		0.38	0.40
C9YTS9	SCAB_67181		0.23	0.30
C9Z6S2	SCAB_27611		0.13	0.13
C9ZHB0	SCAB_66321		0.30	0.26
C9Z4A2	SCAB_58301		0.29	0.21
C9ZHE9	SCAB_66721		0.54	0.42

C9YWC3	SCAB_37401	0.53	0.27
C9Z4V1	SCAB_87931	0.13	0.35
C9ZGX1	SCAB_34721	0.30	0.20
C9YWN2	SCAB_68851	0.19	0.22
C9Z7H6	SCAB_60191	0.07	0.08
C9YVV6	SCAB_20801	0.23	0.16
C9Z4H0	SCAB_73871	0.28	0.13
C9ZCE7	SCAB_63371	0.14	0.15
C9YZ82	SCAB_38641	0.08	0.09
C9ZAJ4	SCAB_45671	0.29	0.21
C9Z723	SCAB_43511	0.38	0.22
C9Z6P3	SCAB_12751	0.13	0.10
C9YUT3	SCAB_35761	0.20	0.15
C9ZEV2	SCAB_17551	0.25	0.31
C9ZFR1	SCAB_65491	0.14	0.18
C9Z436	SCAB_42191	0.16	0.14
C9YTZ7	SCAB_67871	0.30	0.23
C9Z0I7	SCAB_24261	0.06	0.10
C9Z481	SCAB_58071	0.14	0.14

C9Z7E2	SCAB_59881	0.11	0.07
C9Z491	SCAB_58181	0.11	0.14
C9YW84	SCAB_37001	0.14	0.13
C9YZJ9	SCAB_55261	0.10	0.06
C9YVH2	SCAB_68471	0.33	0.44
C9Z871	SCAB_13411	0.11	0.08
C9Z565	SCAB_26701	0.25	0.25
C9Z6Z9	SCAB_28401	0.13	0.15
C9ZHG7	SCAB_66901	0.12	0.14
C9Z8D0	SCAB_28591	0.21	0.25
C9ZHJ9	SCAB_80831	0.05	0.05
C9Z0I8	SCAB_24271	0.07	0.06
C9ZBQ8	SCAB_30621	0.13	0.13
C9YXY2	SCAB_53831	0.08	0.11
C9Z0R8	SCAB_39511	0.25	0.25
C9Z567	SCAB_26721	0.10	0.17
C9Z4Y9	SCAB_11251	0.38	0.31
C9ZBT8	SCAB_30941	0.25	0.44
C9ZAX3	SCAB_62551	0.18	0.27

C9ZAJ1	SCAB_45641	0.09	0.06
C9YTS8	SCAB_67171	0.10	0.10
C9ZBZ4	SCAB_46281	0.18	0.23
C9ZEW2	SCAB_17651	0.11	0.13

NSpC: Calculated by dividing the number of spectra (SpC) for a protein by the molecular weight (MW) of the corresponding protein

CHAPTER 4

GROWTH AND ENZYMATIC ABILITY OF COMMON SCAB-INDUCING SPECIES ON CHEMICAL CONSTITUENTS OF POTATO PERIDERM

4.1. Preface

Common scab is a disease that appears on the potato tubers and characterized by its corky brown lesions with variable sizes. Pathogenic streptomycetes are the causal agents of potato common scab. Although *Streptomyces scabies* is the main causal agent of this disease, other *Streptomyces* species can induce the common scab symptoms such as *Streptomyces acidiscabies* and *Streptomyces turgidiscabies*. Both *S. acidiscabies* and *S. turgidiscabies* are considered to be emerging common scab pathogens.

The results of this work showed that *S. scabies* 87.22 seems to be better adapted to its host plant than the emerging pathogens *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8. When the three strains were grown on suberin-enriched potato periderm, *S. scabies* 87.22 showed the highest growth rate. *S. scabies* 87.22 seemed also to be better suited than other *Streptomyces* species to degrade the cellulosic material embedded in the suberized cell walls of potato periderm. Moreover, both *S. acidiscabies* ATCC 49003 *and S. turgidiscabies* Car8 did not seem to be able to utilize hydroxycinnamates contained in suberin. On the other hand, previous work showed the ability of *S. scabies* to retrieve and utilize those aromatic compounds. These data suggest that *S. scabies* 87.22 could be a more efficient saprophyte and pathogen, compared to the other species.

Results of this work are presented in Section 4.2 and submitted to the Canadian Journal of plant pathology, under the title "Growth and enzymatic ability of common scab-inducing species on chemical constituents of potato periderm". The authors of the manuscript are: Mario Khalil, Rebeca Padilla-Reynaud, Lauriane Giroux, Sylvain Lerat, Carole Beaulieu.

The contribution of each author in the manuscript is as follows: MK and CB designed the experiments. MK performed the lab work with the help of RPR who participated in the gene expression experiment. MK designed the primers. LG did the electron microscopy and SL identified the antimicrobial compound. MK analyzed the data. MK and CB wrote the manuscript. CB supervised the project.

4.2. Manuscript of the article

Title: Growth and enzymatic ability of common scab-inducing species on chemical constituents of potato periderm.

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Abstract

Potato periderm acts as a barrier against the entry of pathogens. The periderm is mainly composed of cellulose and suberin. Suberin has two domains; an aliphatic composed of polyesters of fatty acids and an aromatic one composed mainly of ferulic and coumaric acids. This study revealed that *Streptomyces scabies* 87.22, a strain of the predominant causal agent of potato common scab, showed higher growth than the emerging common scab-inducing species when grown alone or in co-culture with *Streptomyces acidiscabies* strain ATCC 49003 and *Streptomyces turgidiscabies* Car8 in suberin-containing medium. A comparative analysis of the three species secretome from culture media supplemented with potato suberin revealed that *S. scabies* 87.22 accumulated more proteins than the other species which are believed to be involved in cellulose and suberin degradation. *S. scabies* 87.22 showed a higher esterase activity in the presence of suberin compared with *S. acidiscabies* strain ATCC 49003 and *S. turgidiscabies* Car8. The higher esterase activity in case of *S. scabies* 87.22 could be an indication of the ability of this bacterium to degrade the aliphatic part of suberin. Cellulase genes were found to be induced in presence of suberin in *S. scabies* 87.22 only. Both *S. acidiscabies* strain ATCC 49003 and *S. turgidiscabies* Car8 exhibited a poor utilization capacity towards *trans*-ferulic and *p*-coumaric acids which are the main constituents of the suberin aromatic domain. On the other hand, a previous study reported the ability of *S. scabies* to efficiently utilize those compounds. This work suggests that *S. scabies* 87.22 is better adapted to the potato periderm than the emerging pathogens which could give it an advantage as a saprophyte and pathogen over *S. acidiscabies* strain ATCC 49003 and *S. turgidiscabies* Car8.

Key words: suberin, potato periderm, common scab-inducing species, *Streptomyces scabies*, *Streptomyces acidiscabies*, *Streptomyces turgidiscabies*.

Introduction

Streptomycetes are aerobic Gram-positive filamentous bacteria belonging to the phylum Actinobacteria. These spore-forming bacteria are characterized by a genome with high guanine and cytosine (G+C) content and exhibit complex morphological development. Spores of *Streptomyces* species germinate and produce germ tubes which grow by tip extension to produce coenocytic hyphae that branch to form a basal mycelium. This vegetative mycelium further develops into an aerial mycelium that will eventually septate to form spores. *Streptomyces* species use these spores for their dispersal and survival (Flärdh and Buttner, 2009). Most *Streptomyces* species are saprophytes and play a significant role in the decomposition of the organic matter due to the production of several extracellular enzymes that are involved in the degradation of cellulose, hemicellulose, and recalcitrant plant polymers such as lignin (Moraïs et al., 2011; Thierie and Penninckx, 2007).

Although most *Streptomyces* species are saprophytic microorganisms, some species can induce plant diseases. The worldwide distributed *Streptomyces scabies* is the main causal agent of potato common scab (Loria et al., 1997). This disease is characterized by corky lesions on the potato tubers. In addition to *S. scabies*, other emerging species, including *Streptomyces acidiscabies* and *Streptomyces turgidiscabies*, can also cause this disease (Loria et al., 2006). The symptoms caused by the emergent species are similar to those induced by *S. scabies*.

The essential virulence factors in the common scab-inducing species is the production of thaxtomins (Beauséjour et al., 1999; Goyer et al., 1998). Thaxtomins are phytotoxic secondary metabolites that inhibit the biosynthesis of cell wall cellulose.

The potato periderm acts as the first physical barrier that protects tubers against infections caused by common scab-inducing species, as well as other plant pathogenic agents (Beaulieu et al., 2016). Potato tuber cell wall is composed mainly of three parts: the middle lamella, consisting mainly of pectins (Jarrige et al., 1995), the primary cell wall containing cellulose microfibrils and the secondary cell wall consisting of cellulose and hemicellulose which is enriched in phenolic compounds such as suberin. Cellulose is a polysaccharide composed of glucose molecules linked together by β-1,4 bonds while suberin is a lipidic biopolymer. Suberin is made of two moities, a polyaliphatic and a polyaromatic domains (Bernards, 2002). The aliphatic domain of suberin consists of fatty acid polyesters with esterified ferulic acids. Suberin aliphatic domain is very similar to cutin in structure (Beisson et al., 2012).

Previous studies showed that *S. scabies* is able to degrade, at least in part, the aliphatic fraction of suberin (Beaulieu et al., 2016) which is covalently linked to the aromatic fraction. The aromatic fraction of suberin is composed of hydroxycinnamic acids such as ferulic and coumaric acids (Bernards, 2002; Bernards et al., 1995).

When grown in the presence of suberized potato periderm cells, *S. scabies* secretome included several enzymes predicted to play a role in lipid and cellulose metabolisms (Komeil et al., 2014). The production of these extracellular enzymes may lead to the degradation of potato periderm and therefore facilitate the penetration and the infection processes (Komeil et al., 2014). At the very least, previous studies demonstrated that *S. scabies* can degrade cellulose (Padilla-Reynaud et al., 2015) as well as the aliphatic part of suberin (Beaulieu et al., 2016). Recently, a suberinase enzyme (sub1) was detected in *S. scabies* which can act specifically on suberin aliphatic part and cutin releasing fatty acids from both substrates (Jabloune et al. Accepted for publication). A previous study had shown also that *S. scabies* was able

to utilize both *trans*-ferulic and *p*-coumaric acids (the two main constituents of the aromatic part of suberin) as carbon sources (Khalil et al. Accepted for publication).

While the enzymatic behavior of *S. scabies* towards suberized tissues has been partly elucidated (Komeil et al., 2014), no such studies have been conducted with other common scab-inducing species. In this study, the ability of three common scab-inducing species (*S. scabies* 87.22, *S. acidiscabies* strain ATCC 49003 and *S. turgidiscabies* Car8) to grow on suberized tissues was compared. Furthermore, the enzymatic behavior of these pathogenic species in the presence of suberized tissues or suberin constituents was analyzed.

Materials and Methods

Culture conditions

Three common scab-inducing strains, *Streptomyces scabies* strain 87.22 (Bukhalid et al., 1998), *Streptomyces acidiscabies* strain ATCC 49003 (Lambert and Loria, 1989) and *Streptomyces turgidiscabies* Car8 (Miyajima et al., 1998) were used in this study. Bacterial inocula were prepared by inoculating 50 mL of yeast malt extract (YME) (4 g L^{-1} glucose, 4 g L^{-1} yeast extract, and 10 g L^{-1} malt extract) with approximately 10^8 spores. The bacteria were then incubated at 30°C with shaking (250 rpm) for 48 h. The bacterial cells were then recovered by centrifugation (3,500×*g*) for 10 min. The collected pellets were washed twice and resuspended in 5 volumes of saline solution (NaCl 0.85%) and the resulting bacterial suspensions were used as inocula in further experiments. These bacterial inocula (100 µL) were added to 50 mL of YME or 50 mL of the control medium (CM) composed of 0.5 g L^{-1} L-asparagine, 0.5 g L^{-1} K_2HPO_4,

0.2 g L^{-1} $MgSO_4 \cdot 7H_2O$, 10 mg L^{-1} $FeSO_4 \cdot 7H_2O$, and 0.05% (w/v) casein hydrolysate (Sigma-Aldrich). When needed, CM was supplemented with *trans*-ferulic acid (60 µM), *p*-coumaric acid (50 µM) or 0.1% (w/v) suberin. The minimum inhibitory concentrations (MIC) of hydroxycinnamates in *Streptomyces* species were determined and the used concentrations in this study were selected to be in the subinhibitory levels. Suberin was purified from potato tubers according to Kolattukudy and Agrawal (1974) with modifications proposed by Komeil et al. (2013). Culture media were adjusted to pH 7.0 and incubated with shaking (250 rpm) at 30°C.

Determination of bacterial growth

S. scabies strain 87.22, *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 were grown alone or in combination in YME or CM supplemented with potato suberin. Their growth curves were determined by an indirect estimation of the amount of DNA in the culture. One ml of culture was sampled periodically (1, 3 ,5 and 7-day growth). Total bacterial genomic DNA was extracted from collected samples using bacterial genomic DNA extraction kit (Qiagen) according to the manufacturer's instructions and the extracted DNA was eluted using 200 µl of the elution buffer. A gene unique to each strain (*sub1* for *S. scabies* 87.22, *IQ63_12560* for *S. acidiscabies* ATCC 49003 and *fas1* for *S. turgidiscabies* Car8) was amplified from the extracted DNA using quantitative real-time polymerase chain reaction (qPCR) using the Mx3000P qPCR system (Agilent Technologies). qPCR reactions were carried out in a final volume of 20 µl containing 2 µl of template DNA, 1 µl (10 µM) of each primer and 10 µl of BrightGreen 2X qPCR MasterMix-Low ROX (abm). The qPCR conditions were 95°C for 10 min followed by 35 cycles at 95 °C for 15 s and 60°C for 30 s. The amount of DNA in the bacterial cultures was determined by constructing a standard curve for each strain using different dilutions of synthetic sequences (Integrated DNA Technologies). Synthetic sequences are listed in Table 4.1. Table 4.2. presents all

designed primers used in this study. The experiment was done in three biological replicates with two technical replicates per sample and was repeated two times.

Table 4.1. Synthetic sequences used in this study.

Strain	Gene	Predicted function of corresponding protein	Sequence length (bp)	Position in genome	Synthetic sequence
S. scabies 87.22	SCAB_78931 (*sub1*)	Suberinase	162	8717487 to 8717648	AAGTCACTGTCCAGCTACAAGGT GAACTATCCCGCCGACCTTTCGC CCACGTCGGCCGCGCAGGGCAA CGCGGATCTGGTGAACCATGTCC GGAGCCAGGCCGCCTCCTGCCC GAACCAGCGTTTCGTTCTCGTCG GTTATTCGCAGGGCGCGAACGTC GTC
S. acidiscabies ATCC 49003	IQ63_12560	Cellulase	261	18082 to 18342	GGGCCGACGCCCGCGACAACTAC AACACCGGGTGGGTGATCCCGGA CGGTCTGACCGCGTCGGACAGCT ACTCGCAGGTCTATTCGGTCGCC GACAGCTATATCAAGTCGTTCCAG TCCAACCTCGGCGCCAACACCGT ACGTCTGCCGATCAATCCGCCCA GCGTCAGCGAGTCCTGGTGGAGC TCCTACCGGGGCGCGATCGACGC GGCGCTCGCCGACGGCATGAAGG TGGTCATCAGCGCCTGGACCGAGA AGACAA
S.turgidiscabies Car8	L7F500 (*fas1*)	Cytochrome P450	137	6738 to 6874	AGACGCTGTTCGGGGCGCCCGCG TACCTCGTGTGCCGTTACGAGGAC GTACGAGAGGTACTCGCCGACCCG GTCCGGTTCAGCAACGCGCGCTCG CCGCTGTTCGGCATAGACAGCGGA GGTGATGTGACGGAGGAG

Table 4.2. Primers used in this study.

Gene assignation	Bacterial strain	Predicted function of corresponding protein	Primer set (5' to 3')
SCAB_78931 (*sub1*)	*S. scabies* 87.22	Suberinase	For:GGCAACGCGGATCTGGTGAA Rev:CGTTCGCGCCCTGCGAATAA
SCAB_24291	*S. scabies* 87.22	Gyrase A (*gyrA*)	For:GGACATCCAGACGCAGTACA Rev:CTCGGTGTTGAGCTTCTCCT
SCAB_16431	*S. scabies* 87.22	Cellulase CelA1	For:CATGAACCAGGCGCAGATA Rev:CCATGTAGACCCAGGTGTTG
SCAB_17001	*S. scabies* 87.22	Cellulase	For:TCGTCCAGCTGGTGATCTA Rev:GTCGATGTACTGCGTCTTGT
SCAB_17011	*S. scabies* 87.22	Cellulase	For:GAGGCGTACAGCTACCTCCTGTG Rev:GTAGAAGGAGTTGGTCGGCTGGTC
SCAB_17021	*S. scabies* 87.22	Cellulase	For:GACACCTACACCTGGAAGAAC Rev:CTTCTCCTTGCGGTTGAAGA
SCAB_36371	*S. scabies* 87.22	Xylanase/Cellulase	For:CTGAGAAGCCCGGGAAATC Rev:CACCCGTCACACACATGAA
SCAB_51081	*S. scabies* 87.22	Cellulase	For:GGCATCAACTGGTTCGGTTTCGAG Rev:TGTTGTAGCCCAGCGACTTCATCT

SCAB_90101	*S. scabies* 87.22	Cellulase	For:TCGAGTTGGTCGCTGAAATG Rev:AAGCGTGCCGTTGTAGTT
SCAB_8871 (*cel1*)	*S. scabies* 87.22	Cellulase	For:ACCACCAGATCCTCAACAAC Rev:TAGCCGGTGACATAGGAGAT
IQ63_12560	*S. acidiscabies* ATCC 49003	Cellulase	For:GCCCGCGACAACTACAA Rev:GTCCAGGCGCTGATGAC
IQ63_25290	*S. acidiscabies* ATCC 49003	Gyrase A (*gyrA*)	For:AGCTGCTCTCGATGAATGTG Rev:TTCTCCTTCGAGGTGAAGTG
IQ63_18720	*S. acidiscabies* ATCC 49003	Cellulase	For:CTGGCCGATGTGGAAGAC Rev:GTTGGCGTGGATGCTGC
IQ63_34115	*S. acidiscabies* ATCC 49003	Cellulase	For:CCGTACCAGGAGTTCAACAA Rev:GCTGTAGAAGTGGAAGGTCAG
L7F500 (*fas1*)	*S. turgidiscabies* Car8	Cytochrome P450	For:GTACCTCGTGTGCCGTTAC Rev:CATCACCTCCGCTGTCTATG

Antagonism assay

Double agar overlay method was used to test the antagonistic activity between *S. scabies* strain 87.22, *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 (Dopazo et al., 1988). Each strain was grown on YME agar plates by spotting a 10^6 cfu ml^{-1} of spore suspension in the center of the plate for 5 days at 30°C. An overlay [3 ml of soft YME agar (3 g/L agar)] containing a culture of one of the two other species was then poured over the YME plates. The overlay was inoculated with 100 µl of a 3-day-old YME broth culture. The double agar plates were incubated at 30°C for 3 days and presence of a growth inhibition zone in the overlay was scored. Three biological replicates were done and the experiment was repeated twice.

Identification of produced antimicrobial compound

S. acidiscabies ATCC 49003 was grown in oat bran broth for 3 days. The obtained culture was centrifuged (3,500×*g*), the supernatant was recovered and acidified to pH 2-3 with HCl 0.1 N. Secondary metabolites were extracted from the supernatant using ethyl acetate and dried by evaporation. One portion of the recovered secondary metabolites was migrated on a thin layer chromatography (TLC; silica gel, F_{254}) plate (*ca*. 5×10 cm) using chloroform:methanol (9:1). Soft agar (10 mL) containing 500 µL of *S. turgidiscabies* Car8 inoculum was poured on the TLC plate and incubated at 30°C. After 2 days, the presence of a growth inhibition zone indicated the approximate *Rf* of the bioactive molecule. More secondary metabolites with this *Rf* were scraped from TLC plates (20×20 cm) and eluted with chloroform:methanol (7:3) (Lerat et al., 2010).

Metabolites were run on Agilent 1260 Infinity reverse-phase high performance liquid chromatography (HPLC) system equipped with a Zorbax SB-C18 column and separated using a fraction collector. The fractions recovered (each containing one molecule) were tested on *S. turgidiscabies* Car8 for growth inhibition. The molecule showing antimicrobial activity was HPLC-purified in larger amounts. High-resolution accurate mass spectra of the unknown compound and fragmentation spectra were recorded on a maXis 3G (ESI-QqTOF) orthogonal mass spectrometer from Bruker Daltonik (Bremen, Germany) using electrospray ionization in negative ion mode. Fragmentation of selected ions was performed at 25 eV with nitrogen collision gas using a collision-induced dissociation cell. Mass spectra were recorded in the range m/z 50–1200 and external calibration was performed using a sodium formate 0.5 mM solution.

Proteomics analysis

S. scabies 87.22, *S. acidiscabies* strain ATCC 49003 and *S. turgidiscabies* strain Car8 were grown in CM supplemented with potato suberin. Extracellular proteins from 7day-old supernatants were recovered by centrifuging the bacterial cultures (3,500×*g*) for 20 min at 4°C. The supernatants were concentrated to a final volume of 500 µL using Amicon® Ultra-15 Centrifugal Filters-3K and the protein solutions were immediately subjected to proteomic analysis.

Extracellular proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis [10% (w/v) SDS-PAGE] and in-gel protein digestion were carried out according to Komeil et al. (2013). Mass spectrometry was conducted at the Proteomics Platform of the Quebec Genomics Center (Quebec City, Canada) using a hybrid quadrupole time-of-flight (QqTOF) (TripleTOF 5600 plus, SCIEX) coupled to a

capillary HPLC for peptide separation via a nanospray ionization source. All MS/MS spectra were then interpreted using Mascot (Matrix Science, London, UK) to search *S. scabies* 87.22, *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 Uniref100 database to provide statistically validated matches between observed spectra and identified peptides. The results were then uploaded to the scaffold software program (version Scaffold 4.8.8, Proteome Software, Portland, OR, USA) and a filter was set with a 95% minimum protein ID probability with a minimum number of two unique peptides, in which the cut-offs for peptide thresholds were set to 1.0 % false discovery rate (FDR). Protein function was predicted using the UniProt, NCBI, KEGG, and COG databases. The localization of identified proteins was determined by SignalP, TatFind or TatP analysis. The normalized spectral count (NSpC) of proteins was obtained by dividing the number of spectra (SpC) for a protein by the molecular weight (MW) of the corresponding protein and the normalized spectral abundance factor (NSAF) of a protein was calculated by dividing NSpC by the NSpC sum of all proteins in a sample (Neilson et al., 2011). Two replicates for each treatment were done.

Gene expression

S. scabies strain 87.22 and *S. acidiscabies* strain ATCC 49003 were grown in CM supplemented or not with suberin (S) for 5 days. The expression of different genes coding for potential cellulases was tested as follows. By the end of the incubation period, 10 mL of each culture medium were sampled and mixed with 2 mL of stop solution (ethanol/acidic phenol, 95:5, [v/v]) to prevent RNA degradation (Joshi et al., 2007). Bacterial cell pellets were recovered by centrifugation at 4°C (10 min at 3,500×*g*) and stored at –80°C until further use. RNA was extracted from cells using the RNeasy Mini kit (Qiagen) (Lerat et al., 2010). Two (2) µg of total isolated RNA were then reverse transcribed to cDNA using the First strand cDNA synthesis kit (Thermo Scientific Maxima) according to the manufacturer's instructions. Diluted

cDNA (10×) was used to perform quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR). Reactions were carried out in a final volume of 20 µl containing 2 µl of 10x diluted cDNA, 1 µl (10 µM) of both forward & reverse primer and 10 µl of BrightGreen 2X qPCR MasterMix-Low ROX. Primers used for the amplification of the cellulase genes as well as the ones of the reference genes are listed in Table 4.2. The qPCR conditions were 95°C for 10 min followed by 35 cycles at 95 °C for 15 s and 60°C for 30 s. Relative expression levels were determined by using the comparative C_T values according to Pfaffl (2001) with *gyrA* gene of *S. scabies* 87.22 or *S. acidiscabies* ATCC 49003, as a reference genes which was previously validated as a stable reference gene. The experiment was done in four biological replicates with two technical replicates per sample and was repeated two times.

Electron microscopy

S. scabies 87.22, *S. acidiscabies* strain ATCC 49003 and *S. turgidiscabies* Car8 were grown in CM supplemented with suberin for 60 days. After each 10 days of incubation a fraction of the culture supernatant was replaced with fresh CM by centrifugation of the cultures (3,500×*g*) for 20 min, discarding two-thirds of supernatant and then resuspending the pellet in the remaining supernatant to avoid the nutrients depletion. After that, fresh CM was added to the culture to reach the original volume. The incubation was then resumed under the same conditions. The suberin-enriched potato periderm that had been incubated in the presence of *S. scabies* 87.22, *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 for 60 d was fixed in 2.5% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.0). Samples were post-fixed in 1% (w/v) osmium tetroxide in the same buffer. They were dehydrated through a series of graded ethanol (for 5–15 min at each concentration): 70%, 85%, 95%, and 100% three times; and treated three times with propylene oxide. Samples were then infiltrated and

coated with Spurr resin and cut into ultrathin sections. Coloration was performed with lead citrate and uranyl acetate. Samples were observed using the transmission electron microscope Hitachi H-7500 at 80 kV. Potato periderm without bacterial inoculum was used as a control (Beaulieu et al., 2016).

Esterase activity assay

Esterase activities in common scab-inducing culture supernatants were determined using *p*-nitrophenyl butyrate (Sigma-Aldrich) as a substrate (McQueen and Schottel, 1987). The three common scab-inducing species were grown individually in CM supplemented with potato suberin for 5 days. At the end of the incubation period, 300 µl of culture supernatant of the three species was mixed with 690 µl of 20 mmol·l^{-1} Tris buffer, pH 7.5, and 10 µl of a 0.4 mmol·l^{-1} *p*-nitrophenyl butyrate solution. The mixture was incubated at 40°C for 45 min. The increase in the absorbance at 420 nm was read using a spectrometer (Ultrospec 3000-Biochrom) against a blank without culture supernatant. One unit of enzyme activity was defined as the amount of enzyme liberating 1 µmol of *p*-nitrophenol per minute under the assay conditions. The experiment was done in three biological replicates and was repeated two times.

Utilization of cinnamic acids

The ability of *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 to degrade *trans*-ferulic and *p*-coumaric acids was demonstrated using HPLC (as described above) for measuring the residual amounts of both substrates in a CM culture supplemented or not with *trans*-ferulic or *p*-coumaric acids. For each treatment, cultures were grown in triplicate while non inoculated cultures were used as controls.

500 µL of each culture was sampled periodically. The collected samples were filtered through 0.2 µm syringe filters prior to injection in the device. HPLC was used to determine the residual amount of *trans*-ferulic and *p*-coumaric acids. HPLC was carried out using a 25 to 75% acetonitrile linear gradient at a flow rate of 1 ml min^{-1} for 10 min. The absorbance of both substrates was measured at 310 nm and quantified using standard curves which were constructed by using different concentrations of standard stock solutions of both *trans*-ferulic acid and *p*-coumaric acids. The experiment was done in three biological replicates and was repeated two times.

Results

Growth curve of bacterial species

The growth curve of the three common scab-inducing species was determined over a period of 7 incubation days. When the three species were grown individually in YME medium, the amount of DNA recovered varied between 8.3 units and 9.7 units over time and the curve only slightly varied within the three species (Fig. 4.1. D). A lower growth was observed for the three species in CM+S (Fig. 4.1. A). *S. scabies* 87.22 showed the highest growth rate in CM+S while *S. turgidiscabies* Car8 exhibited the lowest growth rate (Fig 4.1. A).

In YME medium, *S. scabies* 87.22 and *S. acidiscabies* ATCC 49003 exhibited a similar growth rate when grown individually or in coculture. In contrast, *S. turgidiscabies* Car8 showed a sharp decline of growth when cocultivated in the presence of the other two species (Fig. 4.1. E). No such growth inhibition was observed when *S. turgidiscabies* Car8 was cocultured in the presence of strain 87.22 only (Fig. 4.1. F). When the three

species were grown together in CM+S (Fig. 4.1. B), the growth of *S. scabies* 87.22 in the co-culture (Fig. 4.1. B) was similar to its growth in the pure culture (Fig. 4.1. A). It is not the case for *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 that showed a lower growth rate in CM+S when grown in coculture (Fig. 4.1. B) than individually (Fig. 4.1. A).

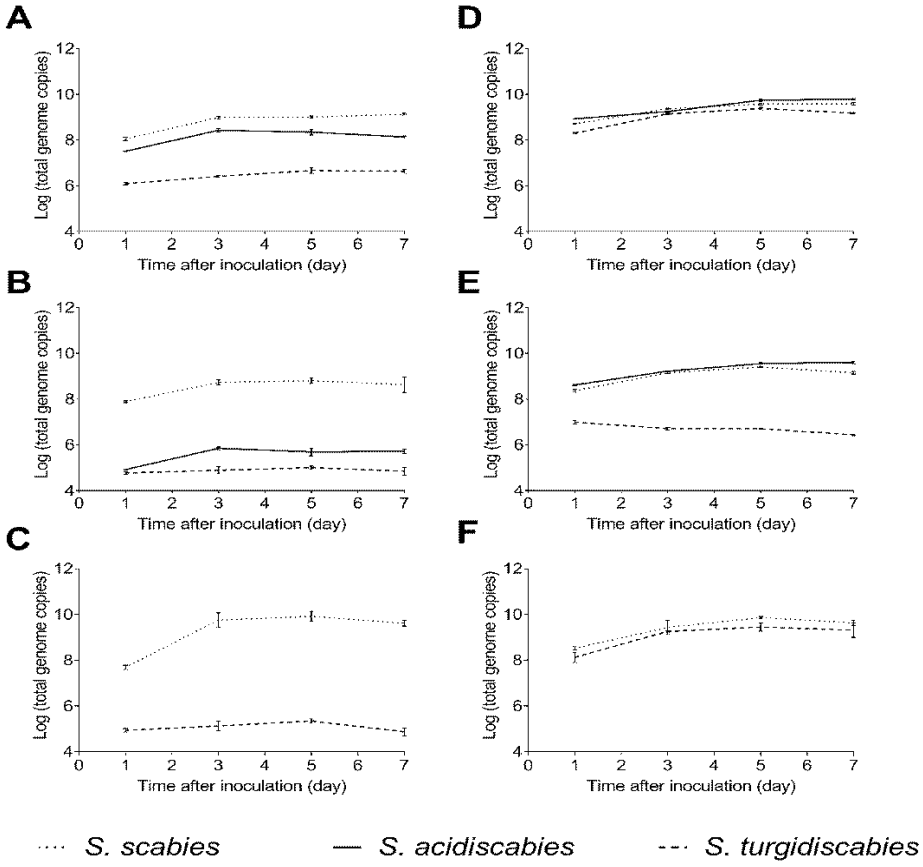

Figure 4.1. Growth of three common scab-inducing species estimated by the number of genomic DNA copies. (A) Pure culture of each species in CM+suberin. (B) Coculture of the three bacterial species in CM+suberin. (C) Coculture of *S. scabies* 87.22 and *S. turgidiscabies* Car8 in CM+suberin. (D) Pure culture of each species in YME. (E) Coculture of the three bacterial species in YME. (F) Coculture of *S. scabies* 87.22 and *S. turgidiscabies* Car8 in YME. Data shown are the mean of three biological replicates. Vertical bars represent standard deviation.

Antagonism between the common scab-inducing species

The antagonism between *S. scabies* strain 87.22, *S. acidiscabies* strain ATCC 49003 and *S. turgidiscabies* Car8 was tested on YME. *S. acidiscabies* ATCC 49003 inhibited the growth of *S. turgidiscabies* Car8 as detected by a clear zone surrounding the colonies of *S. acidiscabies* ATCC 49003 (Fig. 4.2). No antagonism was observed between *S. scabies* 87.22 and *S. turgidiscabies* Car8 or between *S. scabies* 87.22 and *S. acidiscabies* ATCC 49003.

Figure 4.2. Growth inhibition of *S. turgidiscabies* Car8 on YME medium by *S. acidiscabies* ATCC 49003 (colony at center).

Characterization of the antimicrobial compound produced by *S. acidiscabies* ATCC 49003.

Interpretation of ESI mass spectra and fragmentation spectra strongly suggested that the unknown molecule produced by *S. acidiscabies* and showing antimicrobial activity against *S. turgidiscabies* Car8 is oxanthromycin (Supplementary Figure F1).

Secretome analysis of *S. scabies* 87.22, *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 in suberin-containing media

A large amount of the proteins that was found in the supernatants of the three *Streptomyces* species were predicted to have an extracellular localization. The proportion of predicted extracellular proteins in the supernatant was of 49%, 26% and 18% for *S. scabies* 87.22, *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car, respectively.

A total of 195 predicted extracellular proteins were produced by *S. scabies* 87.22 when grown in the presence of suberin while *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 produced 154 and 29 predicted extracellular proteins, respectively. The supplementary Tables (S2, S3 and S4) present the list of these proteins for the three species, respectively. These proteins were divided into 15 functional groups and their NSAF was determined (Table 4.3).

The secretome of *S. scabies* 87.22 is characterized by a predominance of proteins belonging to the functional groups Carbohydrate transport and metabolism,

Replication, transcription and translation and Amino acid transport and metabolism that comprised 55 (NSAF=24.5%), 31 (NSAF=19.8%) and 34 (NSAF=16.4%) proteins, respectively (Table 4.3). Amino acid transport and metabolism represented the largest part of the secretome of *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 with NSAF of 26.7% and 38.9%, respectively. However, in *S. acidiscabies* ATCC 49003 culture, the highest protein diversity was associated with the Carbohydrate transport and metabolism group (52 proteins).

The proteins of the Carbohydrate transport and metabolism group are listed in Table 4.4. Within this group, 30 proteins of *S. scabies* 87.22 were putative glycosyl hydrolases and seven out of 30 were predicted to be cellulases (Table 4.4). The secretome of *S. acidiscabies* ATCC 49003 comprised 26 putative glycosyl hydrolases including two putative cellulases (Table 4.4). Only one protein belonging to Carbohydrate transport and metabolism was detected in the secretome of *S. turgidiscabies* Car8.

Few proteins were associated with lipid metabolism (5 for *S. scabies* (NSAF=2.8%), 6 for *S. acidiscabies* ATCC 49003 (NSAF=4.7%) and one for *S. turgidiscabies* Car8 (NSAF=3.4%) (Table 4.5).

Table 4.3. Predicted extracellular proteins distributed within functional groups of *Streptomyces* species grown in presence of suberin

Functional group	*S. scabies* 87.22	*S. acidiscabies*	*S. turgidiscabies*
	NSAF (%) [number of proteins]	NSAF (%) [number of proteins]	NSAF (%) [number of proteins]
Carbohydrate transport and metabolism	24.48 [55]	24.55 [52]	4.15 [2]
Amino acid transport and metabolism	16.38 [34]	26.73 [27]	38.91 [7]
Lipid transport and metabolism	2.86 [5]	4.69 [6]	3.38 [1]
Energy production and conversion	6.03 [11]	4.94 [5]	21.55 [8]
Cell envelope biogenesis	4.69 [5]	6.21 [6]	2.09 [1]
Replication, transcription and translation	19.76 [31]	0.45 [1]	4.20 [2]
Nucleotide transport and metabolism	0.93 [2]	2.25 [4]	0.00 [0]
Inorganic ion transport and metabolism	2.27 [4]	2.55 [7]	3.15 [1]
Defense mechanism and stress response	4.40 [11]	2.24 [4]	0.00 [0]
Posttranslational modification, protein turnover, Chaperones	1.07 [4]	0.00 [0]	0.00 [0]
Coenzyme transport and metabolism	0.69 [2]	0.73 [1]	5.77 [1]
Secondary metabolism and differentiation	0.35 [2]	0.00 [0]	0.00 [0]
General function prediction only	2.19 [8]	4.50 [9]	0.98 [1]
Function unknown	13.92 [21]	20.16 [32]	15.81 [5]
Total	100 [195]	100 [154]	100 [29]

NSAF: calculated by dividing NSpC of a protein by the NSpC sum of all proteins in a sample.

Table 4.4. Predicted extracellular proteins involved in polysaccharides catabolism of *Streptomyces* species

Uniprot accession number	Corresponding gene	Putative protein function	NSAF (%)
Cellulases			
S. scabies 87.22			
C9ZEQ0	SCAB_17011	Cellulase	0.40
C9YUZ2	SCAB_36371	Xylanase/Cellulase	0.72
C9ZEP9	SCAB_17001	Cellulase	0.60
C9YTK2	SCAB_51081	Cellulase	0.24
C9ZEQ1	SCAB_17021	Cellulase	0.09
C9ZD50	SCAB_16431	Cellulase CelA1	0.36
C9Z9L7	SCAB_90101	Cellulase	1.11
Total			3.52
S. acidiscabies ATCC 49003			
A0A0L0K6F8	IQ63_18720	Cellulase	1.26
A0A0L0JRQ5	IQ63_34115	Cellulase	0.06
Total			1.32
Other Glycosyl hydrolases			
S. scabies 87.22			
C9Z507	SCAB_11431	Glycosyl hydrolase	0.46

C9ZD59	SCAB_16521	Arabinofuranosidase	1.62
C9ZE95	SCAB_79251	Xylanase A	0.95
C9Z2W0	SCAB_72801	Glycosyl hydrolase	0.87
C9Z737	SCAB_43661	Galactan endo-1.6-β-galactosidase	0.52
C9ZCR4	SCAB_78891	Glycosyl hydrolase	0.30
C9YT63	SCAB_19561	β-fructofuranosidase	0.61
C9YVP9	SCAB_6021	β-xylanase	0.75
C9Z4J7	SCAB_74141	α-N-furanosidase	0.27
C9YUL1	SCAB_19941	Arabinofuranosidase	0.45
C9ZE94	SCAB_79241	Arabinofuranosidase	0.39
C9ZFW2	SCAB_66021	β-xylosidase	0.38
C9ZAZ8	SCAB_77201	Glycosyl hydrolase	0.25
C9YU29	SCAB_82021	β-mannosidase	0.34
C9YWP7	SCAB_69011	Lytic transglycosylase	0.24
C9Z1I5	SCAB_85231	Chitinase	0.41
C9ZEB7	SCAB_79481	Xylanase A	0.15
C9ZEC5	SCAB_79561	Glycosyl hydrolase	0.25
C9YVN3	SCAB_5851	Glycosyl hydrolase	0.28
C9Z885	SCAB_13561	Chitobiase	0.06
C9Z7A0	SCAB_44311	α-galactosidase	0.16
C9Z8E5	SCAB_28731	Chitinase C	0.25
C9YYV2	SCAB_22931	Arabinofuranosidase	0.22

C9YUC5	SCAB_4961	Glucuronoarabinoxylan endo-1,4-β-xylanase	0.18
C9Z623	SCAB_74681	Licheninase	0.43
C9ZFW3	SCAB_66031	Arabinofuranosidase	0.21
C9YW88	SCAB_37051	Xylanase	0.30
C9Z2V1	SCAB_72711	Endo-1,4-β -xylanase	0.30
C9ZBE6	SCAB_0631	α-L-fucosidase	0.12
C9ZAH4	SCAB_45451	Glycosylase	0.16
Total			11.88
S. acidiscabies ATCC 49003			
A0A0L0JY42	IQ63_28710	Glycosyl hydrolase	0.38
A0A0L0KCJ5	IQ63_14720	Chitinase	0.70
A0A0L0KQP3	IQ63_00675	Endoglucanase	0.18
A0A0L0JRU5	IQ63_33610	α-L-arabinofuranosidase	0.08
A0A0L0JGB4	IQ63_42510	Glucanase	0.33
A0A0L0JF95	IQ63_42520	Xyloglucanase	0.12
A0A0L0K5M1	IQ63_20970	Rhamnogalacturonase B	0.47
A0A0L0K580	IQ63_21020	Endo-polygalacturonase	0.19
A0A0L0JVB1	IQ63_30610	Arabinan endo-1,5-alpha-L-arabinosidase	0.25
A0A0L0KD81	IQ63_13845	β-xylanase	0.13
A0A0L0JYE0	IQ63_28600	β-xylanase	0.16
A0A0L0JRB2	IQ63_34135	Glucuronoxylanase	0.18
A0A0L0JFE4	IQ63_42515	Cellulose 1,4-beta-cellobiosidase	0.15

A0A0L0JGV2	IQ63_41670	Glycosyl hydrolase	0.34
A0A0L0K636	IQ63_20720	α-arabinofuranosidase	0.36
A0A0L0JZR9	IQ63_27705	β-xylanase	0.15
A0A0L0KH97	IQ63_09940	α-fucosidase	0.28
A0A0L0JXV0	IQ63_28990	Arabinofuranosidase	0.46
A0A0L0JRU4	IQ63_33600	Glucanase	0.22
A0A0L0JD89	IQ63_44720	α-L-fucosidase	0.27
A0A0L0KL29	IQ63_05255	β-1,6-galactanase	0.64
A0A0L0JRQ8	IQ63_34515	α-galactosidase	0.64
A0A0L0JTS1	IQ63_32515	Glycosyl hydrolase	0.73
A0A0L0K6R2	IQ63_18935	Glucan endo-1,3-beta-glucosidase	0.34
A0A0L0KNA7	IQ63_03815	Glycosyl hydrolase	0.54
A0A0L0JNN6	IQ63_35395	Glycosyl hydrolase	0.15
Total			8.44
S. turgidiscabies Car8			
L7FKW2	STRTUCAR8_05433	Glucan endo-1,3-beta-glucosidase	2.00
Total			2.00

NSAF: calculated by dividing NSpC of a protein by the NSpC sum of all proteins in a sample.

Table 4.5. Predicted extracellular proteins involved in lipid metabolism of *Streptomyces* species

Uniprot accession number	Corresponding gene	Putative protein function	NSAF (%)
S. scabies 87.22			
C9ZCR0	SCAB_78851	Sphingolipid ceramide N-deacylase	0.35
C9Z6Y6	SCAB_28271	Cholesterol esterase	1.32
C9ZG71	SCAB_3021	Triacylglycerol lipase	0.49
C9Z5Z2	SCAB_74351	Glycerophosphoryl diester phosphodiesterase	0.37
C9YYE5	SCAB_70541	Lipolytic enzyme	0.29
Total			2.82
S. acidiscabies ATCC 49003			
A0A0L0KIC3	IQ63_09235	Triacylglycerol lipase	0.26
A0A0L0KKU2	IQ63_07390	Glycerophosphodiester phosphodiesterase	0.27
A0A0L0K4E8	IQ63_21355	Cholesterol esterase	0.18
A0A0L0JN14	IQ63_35530	Glycerophosphodiester phosphodiesterase	0.39
A0A0L0KH73	IQ63_09765	Lipase	3.22
A0A0L0JYV2	IQ63_28155	Phosphoric diester hydrolase	0.35
Total			4.67
S. turgidiscabies Car8			
L7F7R8	STRTUCAR8_07544	Acetyl-CoA C-acetyltransferase	3.38
Total			3.38

NSAF: calculated by dividing NSpC of a protein by the NSpC sum of all proteins in a sample.

Expression of cellulase-encoding genes in *S. scabies* 87.22 and *S. acidiscabies* ATCC 49003

The expression of the seven cellulase-encoding genes of *S. scabies* 87.22 that their corresponding proteins were found in the proteome was compared in a control medium supplemented or not with suberin. Gene expression was significantly higher in CM supplemented with suberin (CM+S) than in the control medium for all cellulase genes tested in *S. scabies* 87.22. These cellulase genes were overexpressed in the presence of suberin between 10- and 130-fold (Table 4.6). In contrast, the two cellulase genes of *S. acidiscabies* ATCC 49003 were not upregulated in CM+S (Table 4.6). *S. turgidiscabies* Car8 was not included in the experiment as no cellulases were detected in its secretome in presence of suberin.

Table 4.6. Relative expression level of targeted cellulase genes.

Cellulase gene	Relative gene expression [a](standard deviation)
	CM+S[b]
S. scabies gene	
SCAB_16431	10.27 (0.79) *[c]
SCAB_17001	36.40 (4.15) *
SCAB_17011	131.05 (61.05) *
SCAB_17021	3.82 (0.35) *
SCAB_36371	16.36 (0.80) *
SCAB_51081	56.65 (11.84) *
SCAB_90101	68.18 (5.57) *
S. acidiscabies gene	
IQ63_18720	3.28 (1.72)
IQ63_34115	1.52 (0.35)

Cellulase genes of *S. scabies* 87.22, *S. acidiscabies* ATCC 49003, grown in a control medium supplemented with suberin (CM+S).

[a] Values are the mean of four replicates. Data were normalized with the *gyrA* gene used as internal control which was previously validated as a stable reference gene.

[b] Ratio between gene expression in a control medium containing casein as carbon source and the same medium supplemented with suberin (CM+S).

[c] Values accompanied with an asterisk significantly differ from the control medium ($P<0.05$; LSD test).

Modifications in potato periderm during incubation in the presence of common scab-inducing strains

After a 60-d incubation period, the ground potato periderm incubated with *S. scabies* 87.22, *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 was observed using transmission electron microscopy. The polysaccharides in potato periderm appeared to be markedly different when incubated with the different strains. It was visually observed that the potato periderm contained a significant amount of polysaccharides in presence of *S. turgidiscabies* and in absence of any bacterial inoculum (control medium). However, these polysaccharides in the potato periderm were markedly reduced (less granular consistency) in the presence of both *S. scabies* 87.22 and *S. acidiscabies* ATCC 49003 (Fig. 4.3).

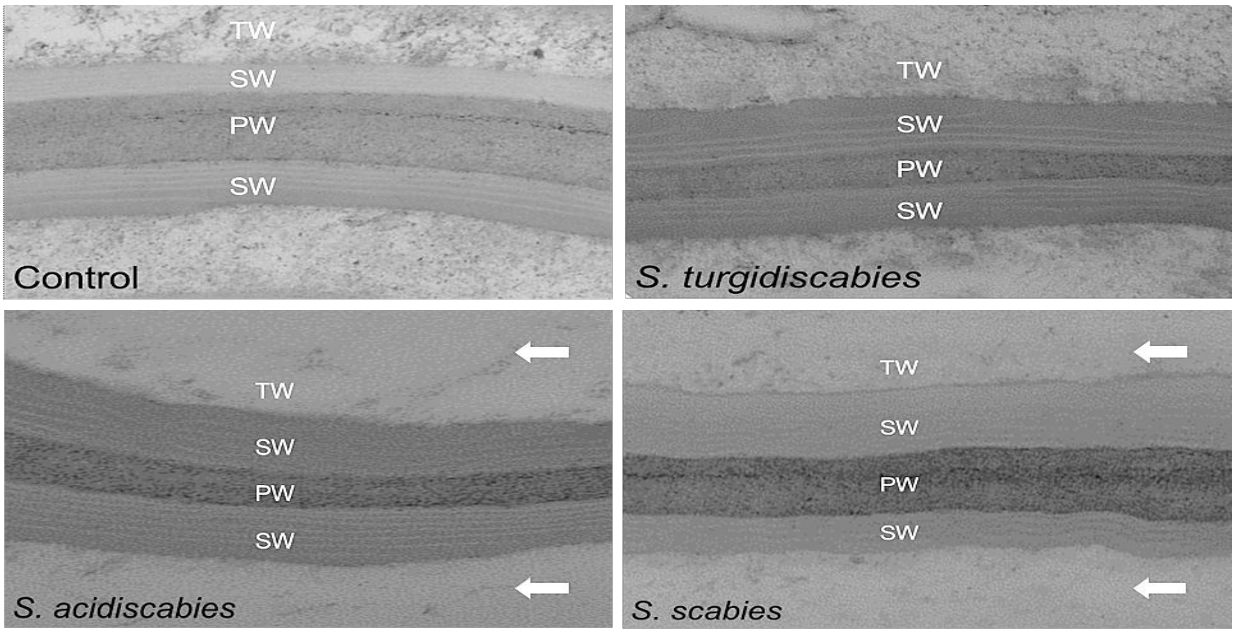

Figure 4.3. Transmission electron micrographs of potato periderm incubated in the presence of common scab-inducing strains. PW: primary cell wall; SW: suberized secondary cell wall; TW: tertiary cell wall. White arrows show degraded polysaccharides (less granular consistency) in both *S. scabies* 87.22 and *S. acidiscabies*.

Esterase activity in common scab-inducing strains

The esterase activity associated with CM+S culture supernatants was compared within the common scab-inducing strains. *S. scabies* 87.22 exhibited the highest esterase activity (22.57 ± 3.64 μmol.min^{-1}.mL^{-1}) followed by *S. acidiscabies* ATCC 49003 (13.43 ± 2.76 μmol.min^{-1}.mL^{-1}) and *S. turgidiscabies* Car8 showed the lowest activity (4.21 ± 0.95 μmol.min^{-1}.mL^{-1}).

Utilization of cinnamic acids by *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8

The time course study of both *trans*-ferulic and *p*-coumaric acids depletion was carried out by sampling *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 cultures up to 72 h. Both strains exhibited a poor utilization capacity towards the two substrates. *S. acidiscabies* ATCC 49003 showed higher degradation affinity towards *p*-coumaric acid when compared to *trans*-ferulic acid. After 72 h of incubation, *S. acidiscabies* ATCC 49003 consumed about 8.3% and 21% of the added *trans*-ferulic and *p*-coumaric acid, respectively. On the other hand, *S. turgidiscabies* Car8 consumed about 7.5% and 9% of the added *trans*-ferulic and *p*-coumaric acids, respectively (Fig. 4.4).

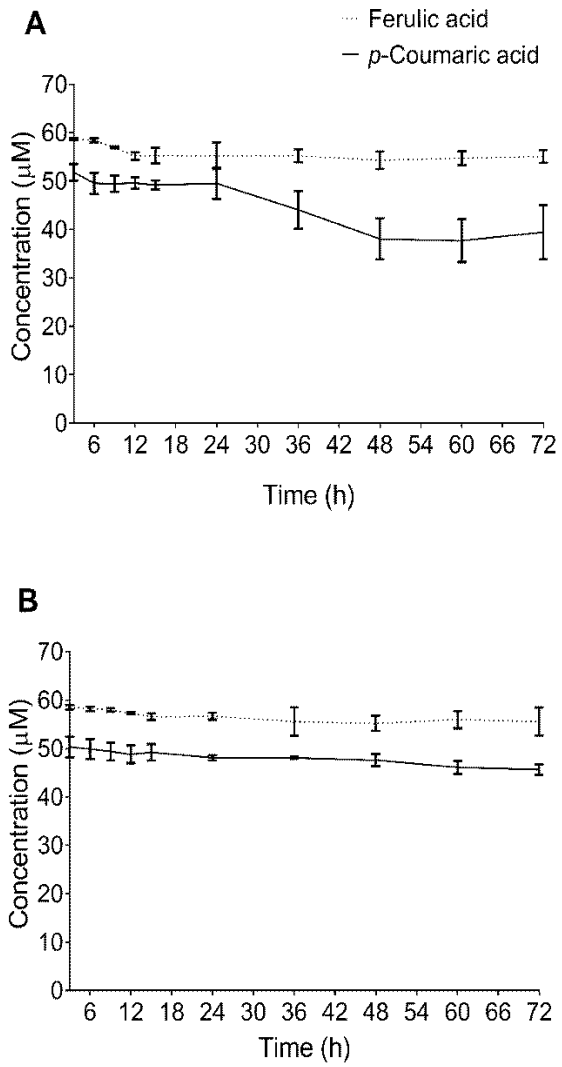

Figure 4.4. Kinetics of *trans*-ferulic and *p*-coumaric acids utilization in *S. acidiscabies* ATCC49003 (A) and *S. turgidiscabies* Car8 (B). Data shown are the mean of three biological replicates. Vertical bars (in curves) representing standard deviation.

Discussion

In this work, three phylogenetically distant common scab-inducing species found in North America (*S. scabies* 87.22, *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8) were compared. While the catabolic ability of these common scab-inducing species has been determined on some carbon sources (Lambert and Loria, 1989a, 1989b; Miyajima et al., 1998), these causal agents have not been compared for their ability to consume periderm constituents or to propagate on potato periderm, the outer layer of potato tuber. The three common scab-inducing species used in this study showed a very similar growth pattern in a rich medium such as YME (this study), but in a medium containing suberin-enriched potato periderm as main carbon source, *S. scabies* 87.22 exhibited a higher growth rate than both *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 when grown as pure culture on suberin-enriched potato periderm. A previous chemical analysis of potato phellem exposed to the presence of *S. scabies* demonstrated the ability of *S. scabies* to release sugars and lipidic compounds from suberin-enriched phellem (Beaulieu et al., 2016). The fact that the growth of *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 declined when they were cocultured with *S. scabies* 87.22 on suberized tissues but not in YME reinforced the assumption that *S. scabies* 87.22 utilized more efficiently than the two other pathogens the constituents of potato periderm.

The difference in growth rates in coculture could not be only attributed to competition for nutrients, but also to production of antimicrobial compounds. The growth rate of *S. turgidiscabies* Car8 declined when it was co-cultured with the two other species in both yeast malt extract and suberin media. The antagonism assay revealed the ability of *S. acidiscabies* ATCC 49003 to inhibit the growth *S. turgidiscabies* Car8. The inhibited growth of *S. turgidiscabies* Car8 was due to the production by *S. acidiscabies* ATCC 49003 of a rare class of polyketides, the oxanthromicin. This polyketide was

isolated previously from rare actinobacterial species (Patel et al., 1984; Salim et al., 2014). This antibiotic was also shown to modulate K-Ras plasma membrane localization (Salim et al., 2014). Ras GTPases are key molecular switches that regulate cell growth, proliferation and differentiation, and are ubiquitously expressed in mammalian cells as three isoforms (H-Ras, N-Ras and K-Ras) (Salim et al., 2014). Ras GTPases have been also detected in plants but not the K-Ras isoforms (Vernoud et al., 2003). Nevertheless, further studies are required to investigate the potential role of oxanthromicin produced by *S. acidiscabies* ATCC 49003 in the process of pathogenicity. As no apparent antagonism was observed between *S. scabies* 87.22 and *S. turgidiscabies* Car8, the occurrence of both *S. scabies* 87.22 and *S. turgidiscabies* Car8 in the same potato field and even in the same common scab lesion can be explained (Lehtonen et al., 2004).

The assumption that *S. scabies* 87.22 utilized more efficiently than the two other pathogens the constituents of potato periderm is supported by the fact that the proportion of predicted extracellular proteins was markedly higher in its suberin-containing culture supernatant compared to that of *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8. The higher concentration of predicted intracellular proteins of *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 in the supernatant of suberin-enriched medium could be explained by cell lysis, reflecting their poor ability to obtain carbon from the suberized potato tissues.

It is known that the suberin preparation contains a non-negligible amount of polysaccharides (Padilla-Reynaud et al., 2015). The ability of both *S. scabies* 87.22 and *S. acidiscabies* ATCC 49003 to degrade the polysaccharides associated with suberin has been confirmed by transmission electron microscopy. There was no such

apparent degradation of polysaccharides in *S. turgidiscabies* Car8, which could be due to the presence of a single glycosyl hydrolase in the secretome of this species.

S. scabies 87.22 produced both a higher proportion and a higher diversity of glycosyl hydrolases as well as cellulases on suberized tissues compared to *S. acidiscabies* ATCC 49003. Interestingly, suberin exerts an inducing effect on the cellulase genes expression of *S. scabies* 87.22 (Padilla-Reynaud et al., 2015) but not in *S. turgidiscabies* Car8 (data not shown). In *S. acidiscabies* ATCC 49003 as in most *Streptomyces* species, cellulose degradation is induced by degradation products of cellulose (Hodgson, 2000; Lin and Wilson, 1987; Pérez-pons et al., 1995).

Book et al., (2016) demonstrated a close relationship between the CAZy gene content of both *S. acidiscabies* ATCC 49003 and *S. scabies* 87.22 and highly cellulolytic streptomycetes, suggesting that cellulolytic activity might contribute to pathogenesis. Although no cellulase was detected in the proteomic assay, the genome of *S. turgidiscabies* Car8 also harbors cellulase encoding genes and one cannot exclude that the corresponding enzymes might be produced during tuber infection. The biosynthesis of cellulases is controlled by the CebR repressor in non-pathogenic *Streptomyces* species (Marushima et al., 2009). In *S. scabies*, this repressor was reported to be involved in pathogenicity by modulating thaxtomin synthesis (Francis et al., 2015). CebR is released from its binding sites located upstream of the thaxtomin biosynthetic cluster when it binds to cellobiose allowing transcription of the thaxtomin biosynthetic and regulatory genes (Jourdan et al., 2016). Cellobiose was also identified as an inducer of thaxtomin biosynthetic genes as it binds TxtR, the transcriptional activator of the thaxtomin biosynthetic gene cluster (Joshi et al., 2007). *S. scabies* cellulases could thus be important for common scab-inducing

Streptomyces species by providing cellobiose, the disaccharide that triggers thaxtomin production.

Esterases and lipases are the types of enzymes expected to degrade polyesters such as suberin (Kontkanen et al., 2009). Lipases and esterases may cleave ester bonds within the aliphatic domain of suberin, thereby rendering the periderm more permeable to the abundant and various hydrophilic carbohydrate-degrading enzymes (Beaulieu et al., 2016). *S. scabies* 87.22 accumulated proteins belonging to those classes in presence of suberin. Some of these proteins were identified previously in the secretome of *S. scabies* to be possibly involved in suberin degradation (Komeil et al., 2014). Some proteins involved in lipid metabolism were also detected in the secretome of *S. acidiscabies* ATCC 49003 and one protein involved in lipid metabolism was seen in the secretome of *S. turgidiscabies* Car8. Although *S. acidiscabies* ATCC 49003 produced a higher amount of extracellular esterases and lipases in response to suberin, *S. scabies* 87.22 seems to be more adapted to degrade the lipid part of suberin as it showed a higher esterase activity in the presence of suberin than *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8.

Beaulieu et al. (2016) showed that *S. scabies* has the ability to release cinnamic acids from potato periderm as the concentration of ferulic acid in potato phellem decreased after a prolonged incubation in the presence of *S. scabies*. It has also been shown that *S. scabies* was able to efficiently catabolized *trans*-ferulic and *p*-coumaric acids which are the main constituents of the aromatic part of suberin (Khalil et al. Accepted for publication). While *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 exhibited a very poor utilization capacity towards hydroxycinnamic acids (*trans*-ferulic and *p*-coumaric acids) after 72 hours of incubation, *S. scabies* degraded ferulic and coumaric acids within 15 hours of incubation (Khalil et al. Accepted for publication).

The ability to catabolize aromatic compounds may be an asset for *S. scabies*. In other phytopathogens such as *Fusarium oxysporum* (Michielse et al., 2012), the capability to degrade aromatic compounds has been shown to be related to virulence. Although the relationship between degradation of aromatic compounds and *S. scabies* virulence remains to be investigated, the ability of *S. scabies* to degrade these compounds could contribute to its fitness in the early stages of pathogenesis by protecting it from inhibition by toxic hydroxycinnamic acids produced by plants as a defense mechanism (Lowe et al., 2015).

S. scabies 87.22 appears to be better adapted to its host plant than the emerging pathogens *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8. Previous reports suggested that *S. acidiscabies* does not persist in soil (Lambert and Loria, 1989a), and that *S. turgidiscabies* is very infrequent in North America (Wanner, 2009). The ability of *S. scabies* 87.22 to grow efficiently on potato periderm could help it maintain its populations in soil, as the degradation of suberin is a very slow process in nature (Beaulieu et al., 2016; Hamer et al., 2012) and attributed to rare microorganisms (Kontkanen et al., 2009).

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Table S2. Supplementary Table. Proteins produced by *S. scabies* 87.22 grown in CM+suberin.

Uniprot accession number	Corresponding gene in *S. scabies* 87.22 genome	Putative protein function	NSpC
Carbohydrate transport and metabolism			
C9Z507	SCAB_11431	Glycosyl hydrolase	0.24
C9ZD59	SCAB_16521	Arabinofuranosidase	0.85
C9Z5L1	SCAB_42951	Glucose / Sorbosone dehydrogenase	0.80
C9ZE95	SCAB_79251	Xylanase A	0.50
C9ZEQ0	SCAB_17011	Cellulase	0.21
C9Z2W0	SCAB_72801	Glycosyl hydrolase	0.46
C9Z737	SCAB_43661	Galactan endo-1.6-β-galactosidase	0.28
C9YUZ2	SCAB_36371	Xylanase/Cellulase	0.38
C9ZCR4	SCAB_78891	Glycosyl hydrolase	0.16
C9ZEP9	SCAB_17001	Cellulase	0.32
C9YT63	SCAB_19561	β-fructofuranosidase	0.32
C9Z1U5	SCAB_9381	Exo-α-sialidase	0.32
C9YVP9	SCAB_6021	β-xylanase	0.39
C9YUG2	SCAB_5351	D-ribose-binding periplasmic protein	0.39
C9Z451	SCAB_57751	Cellobiose-binding transport system associated	0.27

C9Z4J7	SCAB_74141	α-N-furanosidase	0.14
C9YUL1	SCAB_19941	Arabinofuranosidase	0.24
C9ZB22	SCAB_77441	α-arabinanase	0.18
C9ZE94	SCAB_79241	Arabinofuranosidase	0.21
C9ZFW2	SCAB_66021	β-xylosidase	0.20
C9ZAZ8	SCAB_77201	Glycosyl hydrolase	0.13
C9Z433	SCAB_42161	Fructose 1.6-bisphosphate aldolase 2.3-bisphosphoglycerate-dependent	0.28
C9Z804	SCAB_89741	Cellulose-binding protein	0.39
C9YU66	SCAB_82411	Pectate lyase	0.33
C9YYF0	SCAB_70591	Pectate lyase	0.23
C9YU29	SCAB_82021	β-mannosidase	0.18
C9YWP7	SCAB_69011	Lytic transglycosylase	0.13
C9Z1T6	SCAB_9291	Lactonase	0.18
C9ZE74	SCAB_79011	Acetyl-xylan esterase	0.18
C9YTK2	SCAB_51081	Cellulase	0.13
C9Z1I5	SCAB_85231	Chitinase	0.22
C9ZEB7	SCAB_79481	Xylanase A	0.08
C9ZEC5	SCAB_79561	Glycosyl hydrolase	0.13
C9ZEQ1	SCAB_17021	Cellulase	0.05

C9ZD50	SCAB_16431	Cellulase	0.19
C9YVN3	SCAB_5851	Glycosyl hydrolase	0.15
C9YY63	SCAB_69711	Phosphoglycerate kinase	0.10
C9Z885	SCAB_13561	Chitobiase	0.03
C9Z7A0	SCAB_44311	α-galactosidase	0.09
C9Z8E5	SCAB_28731	Chitinase C	0.13
C9YYV2	SCAB_22931	Arabinofuranosidase	0.12
C9YUC5	SCAB_4961	Glucuronoarabinoxylan endo-1,4-β-xylanase	0.10
C9Z623	SCAB_74681	Licheninase	0.23
C9ZFW3	SCAB_66031	Arabinofuranosidase	0.11
C9Z9L7	SCAB_90101	Cellulase	0.58
C9YW88	SCAB_37051	Xylanase	0.16
C9YY83	SCAB_69911	Transaldolase	0.16
C9Z2V1	SCAB_72711	Endo-1,4-β -xylanase	0.16
C9ZBE6	SCAB_0631	α-L-fucosidase	0.07
C9YVX8	SCAB_21021	Xylose ABC transporter	0.13
C9ZDW4	SCAB_63891	ABC-type xylose transport-system, periplasmic	0.15
C9YTR6	SCAB_67051	Dihydrolipoyllysine-residue succinyltransferase	0.09
C9YVY6	SCAB_21101	Aconitate hydratase	0.19
C9ZAH4	SCAB_45451	Glycosylase	0.16

C9Z9C8	SCAB_76271	Glycogen accumulation regulator GarA	0.16
Amino acid transport and metabolism			
C9Z204	SCAB_24891	Glutamate uptake system binding subunit	1.07
C9YY61	SCAB_69691	zinc-binding carboxypeptidase	0.29
C9Z5D4	SCAB_27411	Oligopeptide-binding system protein	0.32
C9YWP0	SCAB_68931	Branched-chain amino acid ABC transporter	0.48
C9YXR8	SCAB_37611	Aminopeptidase	0.60
C9ZGG7	SCAB_18081	γ-glutamyltranspeptidase	0.34
C9Z281	SCAB_25691	4-aminobutyrate aminotransferase	0.35
C9YYT5	SCAB_8041	Amidohydrolase	0.11
C9Z1E7	SCAB_72231	Serine protease	0.26
C9Z058	SCAB_84971	Amidase	0.13
C9Z7C0	SCAB_59651	Serine protease	0.08
C9Z7C5	SCAB_59701	Aminopeptidase	0.05
C9YTK4	SCAB_51101	Phosphoserine aminotransferase	0.45
C9YZP9	SCAB_70761	Solute-binding protein	0.27
C9YTR8	SCAB_67071	Cytosol aminopeptidase	0.14
C9YXZ5	SCAB_53981	Peptidase	0.06
C9YZE4	SCAB_54701	Urocanate hydratase	0.11
C9ZHG5	SCAB_66881	Glutamine synthetase	0.08

C9ZBZ2	SCAB_31501	Peptide ABC transporter	0.07
C9Z4N7	SCAB_87271	Peptidase	0.07
C9YWB0	SCAB_37271	Aspartate aminotransferase	0.11
C9Z7C3	SCAB_59681	Peptidase	0.09
C9Z0P2	SCAB_24881	Glutamate uptake system ATP-binding subunit	0.11
C9YVT3	SCAB_6381	Extracellular small neutral protease	0.18
C9Z578	SCAB_26841	Serine protease	0.15
C9Z160	SCAB_56441	protease	0.12
C9Z760	SCAB_43901	Tripeptidyl aminopeptidase	0.61
C9Z862	SCAB_13321	X-prolyl-dipeptidyl aminopeptidase	0.32
C9Z994	SCAB_61611	Protease	0.10
C9YY98	SCAB_70071	Cysteine desulfurase	0.20
C9Z047	SCAB_84861	Amidase	0.04
C9ZCL9	SCAB_78431	Tripeptidylaminopeptidase	0.30
C9YTK3	SCAB_51091	Peptidase	0.26
C9ZAW6	SCAB_62471	Aminopeptidase	0.62
Lipid transport and metabolism			
C9ZCR0	SCAB_78851	Sphingolipid ceramide N-deacylase	0.19
C9Z6Y6	SCAB_28271	Cholesterol esterase	0.70
C9ZG71	SCAB_3021	Triacylglycerol lipase	0.26

C9Z5Z2	SCAB_74351	Glycerophosphoryl diester phosphodiesterase	0.20
C9YYE5	SCAB_70541	Lipolytic enzyme	0.16
Energy production and conversion			
C9Z8F0	SCAB_28781	ATP synthase subunit α	0.58
C9ZE86	SCAB_79151	Cytokinin dehydrogenase	0.45
C9Z1E2	SCAB_72171	Acyl-CoA dehydrogenase	0.08
C9ZGW6	SCAB_34651	Oxidoreductase	0.39
C9YTU0	SCAB_67291	Cytochrome C oxidase subunit II	0.47
C9Z2T7	SCAB_72571	Molybdopterin oxidoreductase	0.09
C9YUX5	SCAB_36201	IMP dehydrogenase/ GMP reductase	0.14
C9ZHB0	SCAB_66321	F420-dependent NADP reductase	0.18
C9Z4H0	SCAB_73871	Dehydrogenase	0.20
C9Z871	SCAB_13411	Oxidoreductase	0.36
C9YTC3	SCAB_35291	Succinate dehydrogenase flavoprotein subunit	0.20
Cell envelope biogenesis			
C9Z240	SCAB_25251	Polyribonucleotide nucleotidyltransferase	0.36
C9YT92	SCAB_34981	lipoprotein	1.31
C9Z8V2	SCAB_45141	D-alanyl-D-alanine carboxypeptidase	0.25
C9YTV5	SCAB_67441	Muramoyltetrapeptide carboxypeptidase	0.10
C9YUC2	SCAB_4931	Murein DD-endopeptidase MepM	0.06

Replication, transcription and translation

C9YW79	SCAB_36951	30S ribosomal protein S10	0.38
C9YW78	SCAB_36941	50S ribosomal protein L3	0.20
C9YW75	SCAB_36911	50S ribosomal protein L2	0.47
C9Z4I0	SCAB_73981	50S ribosomal protein L20	0.79
C9YW70	SCAB_36861	50S ribosomal protein L29	1.25
C9Z656	SCAB_75031	30S ribosomal protein S4	0.35
C9YW76	SCAB_36921	50S ribosomal protein L23	0.43
C9YW46	SCAB_36621	30S ribosomal protein S9	0.32
C9YW73	SCAB_36891	50S ribosomal protein L22	0.46
C9YW47	SCAB_36631	50S ribosomal protein L13	0.38
C9YW68	SCAB_36841	50S ribosomal protein L14	0.42
C9YW77	SCAB_36931	50S ribosomal protein L4	0.24
C9YW95	SCAB_37121	30S ribosomal protein S12	0.32
C9YW72	SCAB_36881	30S ribosomal protein S3	0.15
C9Z3Q3	SCAB_26151	50S ribosomal protein L19	0.38
C9YW62	SCAB_36781	50S ribosomal protein L18	0.25
C9YUT0	SCAB_35731	30S ribosomal protein S17	0.33
C9Z4H9	SCAB_73971	50S ribosomal protein L35	0.43
C9Z241	SCAB_25261	30S ribosomal protein S15	0.50

C9YW64	SCAB_36801	30S ribosomal protein S8	0.21
C9Z7H3	SCAB_60161	50S ribosomal protein L27	0.22
C9YW67	SCAB_36831	50S ribosomal protein L24	0.21
C9Z3P0	SCAB_26021	30S ribosomal protein S2	0.07
C9YW61	SCAB_36771	30S ribosomal protein S5	0.10
C9ZAN4	SCAB_46111	Single-stranded DNA-binding protein	0.32
C9YWA1	SCAB_37181	DNA-directed RNA polymerase subunit β	0.18
C9YWA2	SCAB_37191	DNA-directed RNA polymerase subunit β	0.15
C9YW51	SCAB_36671	DNA-directed RNA polymerase subunit α	0.22
C9YW66	SCAB_36821	50S ribosomal protein L5	0.31
C9Z996	SCAB_61631	Ribonuclease Z	0.10
C9Z7K6	SCAB_75261	30S ribosomal protein S13	0.17
Nucleotide transport and metabolism			
C9ZGX4	SCAB_49491	5'-nucleotidase	0.18
C9YVK8	SCAB_68841	5'-nucleotidase	0.30
Inorganic ion transport and metabolism			
C9Z473	SCAB_57981	Protein DesF, iron transport system	0.28
C9YUK3	SCAB_19841	Aliphatic sulfonate ABC transporter substrate-binding protein	0.67
C9ZH91	SCAB_66141	Alkaline phosphatase	0.10

C9ZB70	SCAB_77971	Phosphatase	0.14
Defense mechanism and stress response			
C9YZ91	SCAB_38731	Beta-lactamase	0.35
C9YWG0	SCAB_53111	Penicillin acylase	0.23
C9YYC2	SCAB_70311	TerD-like stress protein	0.15
C9Z1W0	SCAB_9531	Catalase-peroxidase	0.05
C9Z5G9	SCAB_42541	Chaperone protein DnaK	0.05
C9Z7C8	SCAB_59731	Superoxide dismutase	0.10
C9ZHS9	SCAB_81661	TerD-like stress protein	0.45
C9Z0L9	SCAB_24621	TerD-like stress protein	0.26
C9ZE08	SCAB_64341	TerD-like stress protein	0.28
C9Z233	SCAB_25181	β-lactamase	0.23
C9YVV6	SCAB_20801	Chlorite dismutase	0.14
Posttranslational modification, protein turnover, Chaperones			
C9Z4D5	SCAB_73521	Peptidyl-prolyl cis-trans isomerase	0.09
C9ZAJ2	SCAB_45651	Peptidyl-prolyl cis-trans isomerase	0.16
C9Z0R6	SCAB_39491	Clp-family ATP-binding protease	0.04
C9YUY5	SCAB_36301	10 chaperonin	0.27
Coenzyme transport and metabolism			
C9Z1Y4	SCAB_9771	Cobalamin biosynthesis protein cobN	0.18

C9Z7P0	SCAB_75631	6.7-dimethyl-8-ribityllumazine synthase	0.18
Secondary metabolism and differentiation			
C9ZD95	SCAB_31511	BldKD oligopeptide ABC transporter subunit	0.09
C9ZD97	SCAB_31531	BldKB-like transport system extracellular solute-binding protein	0.09
General function prediction only			
C9Z2V8	SCAB_72781	Penicillin acylase	0.23
C9ZE96	SCAB_79261	Feruloyl esterase	0.11
C9Z770	SCAB_44001	Lysyl oxidase	0.18
C9Z3D2	SCAB_10131	Lectin	0.09
C9Z0C9	SCAB_8801	Subtilase-type protease inhibitor	0.20
C9Z6S2	SCAB_27611	Phage tail sheath proteins	0.24
C9ZGS4	SCAB_34211	Galactose oxidase	0.03
C9YWW9	SCAB_82711	Serine/threonine protein kinase	0.06
Function unknown			
C9ZGQ1	SCAB_33981		1.03
C9YV41	SCAB_52001		1.02
C9YTD0	SCAB_35361		0.88
C9Z1G8	SCAB_72441		0.35
C9ZB66	SCAB_77921		0.28

C9YVU0	SCAB_20641	0.35
C9YVL7	SCAB_5681	0.34
C9ZDY2	SCAB_64081	0.27
C9Z9T4	SCAB_90811	0.32
C9ZBE2	SCAB_0601	0.06
C9Z064	SCAB_85031	0.31
C9YUE9	SCAB_5221	0.21
C9Z9U4	SCAB_90901	0.38
C9Z0X8	SCAB_40131	0.09
C9Z765	SCAB_43951	0.20
C9Z7A3	SCAB_44341	0.17
C9ZHG0	SCAB_66831	0.13
C9ZH08	SCAB_49851	0.14
C9Z6Q2	SCAB_12841	0.10
C9YTI5	SCAB_50911	0.09
C9Z8T0	SCAB_44921	0.07

Table S3. Supplementary Table. Proteins produced by *S. acidiscabies* ATCC 49003 grown in CM+suberin.

Uniprot accession number	Corresponding gene in *S. acidiscabies* ATCC 49003 genome	Putative protein function	NSpC
Carbohydrate transport and metabolism			
A0A0L0JW93	IQ63_30600	Aldose 1-epimerase	1.06
A0A0L0JY42	IQ63_28710	Glycosyl hydrolase	0.18
A0A0L0KCJ5	IQ63_14720	Chitinase	0.33
A0A0L0JHQ3	IQ63_41145	Sugar ABC transporter substrate-binding protein	0.41
A0A0L0KQP3	IQ63_00675	Endoglucanase	0.09
A0A0L0JRU5	IQ63_33610	Alpha-L-arabinofuranosidase	0.04
A0A0L0JGB4	IQ63_42510	Glucanase	0.16
A0A0L0K8H1	IQ63_16975	Acetyl xylan esterase	0.06
A0A0L0KEE6	IQ63_12925	Alpha-amylase	0.04
A0A0L0JJZ4	IQ63_39315	Sugar ABC transporter substrate-binding protein	0.07
A0A0L0JRK2	IQ63_33805	Ricin B lectin	0.04
A0A0L0JQN1	IQ63_34155	Sugar dehydrogenase	0.14
A0A0L0JF95	IQ63_42520	Xyloglucanase	0.06

A0A0L0JSU2	IQ63_32525	Sugar ABC transporter substrate-binding protein	0.11
A0A0L0K5M1	IQ63_20970	Rhamnogalacturonase B	0.22
A0A0L0K580	IQ63_21020	Endo-polygalacturonase	0.09
A0A0L0JJS6	IQ63_40110	Esterase	0.10
A0A0L0JVB1	IQ63_30610	Arabinan endo-1,5-alpha-L-arabinosidase	0.12
A0A0L0KD81	IQ63_13845	Beta-xylanase	0.06
A0A0L0JYE0	IQ63_28600	Beta-xylanase	0.08
A0A0L0JRB2	IQ63_34135	Glucuronoxylanase	0.09
A0A0L0KHD8	IQ63_09395	Levanase	0.09
A0A0L0KPP1	IQ63_02975	Glucose sorbosone dehydrogenase	0.13
A0A0L0KI52	IQ63_09945	Ricin B lectin	0.21
A0A0L0JFE4	IQ63_42515	Cellulose 1,4-beta-cellobiosidase	0.07
A0A0L0JGV2	IQ63_41670	Glycosyl hydrolase	0.16
A0A0L0K636	IQ63_20720	Alpha-arabinofuranosidase	0.17
A0A0L0JZR9	IQ63_27705	Beta-xylanase	0.07
A0A0L0JTR2	IQ63_32455	Hydrolase	0.23
A0A0L0KH97	IQ63_09940	Alpha-fucosidase	0.13
A0A0L0JXV0	IQ63_28990	Arabinofuranosidase	0.22
A0A0L0JRU4	IQ63_33600	Glucanase	0.10
A0A0L0K983	IQ63_16765	Silent information regulator protein Sir2	0.20

A0A0L0JJ32	IQ63_40120	Pectate lyase	0.39
A0A0L0JD89	IQ63_44720	Alpha-L-fucosidase	0.13
A0A0L0JEQ7	IQ63_43105	Pectate lyase	0.36
A0A0L0K5B2	IQ63_20965	Carbohydrate esterase	0.43
A0A0L0KL29	IQ63_05255	Beta-1,6-galactanase	0.30
A0A0L0JVA7	IQ63_30585	Sugar ABC transporter substrate-binding protein	0.46
A0A0L0JG22	IQ63_43090	Pectate lyase	0.45
A0A0L0K6F8	IQ63_18720	Cellulase	0.59
A0A0L0JRQ5	IQ63_34115	Cellulase	0.03
A0A0L0JRQ8	IQ63_34515	Alpha-galactosidase	0.30
A0A0L0K577	IQ63_21725	ABC transporter substrate-binding protein	0.20
A0A0L0KLR3	IQ63_04750	ABC transporter substrate-binding protein	0.16
A0A0L0JTS1	IQ63_32515	Glycosyl hydrolase	0.73
A0A0L0K5Q7	IQ63_20870	Extracellular solute-binding protein	0.08
A0A0L0K7L0	IQ63_18560	Beta-N-acetylhexosaminidase	0.03
A0A0L0JFR3	IQ63_43085	Rhamnogalacturonan acetylesterase RhgT	0.53
A0A0L0KNA7	IQ63_03815	Glycosyl hydrolase	0.54
A0A0L0K6R2	IQ63_18935	Glucan endo-1,3-beta-glucosidase	0.34
A0A0L0JNN6	IQ63_35395	Glycosyl hydrolase	0.15

Amino acid transport and metabolism

A0A0L0KME3	IQ63_05610	Protease	0.52
A0A0L0K6S1	IQ63_18490	Protease	0.43
A0A0L0KKN0	IQ63_08505	ATP-dependent zinc metalloprotease FtsH	0.30
A0A0L0KNI6	IQ63_04740	Tripeptidyl aminopeptidase	0.42
A0A0L0KLQ2	IQ63_07710	Peptidase M14	0.14
A0A0L0JJY2	IQ63_37820	Esterase	0.10
A0A0L0JQS0	IQ63_34320	Amidohydrolase	0.14
A0A0L0KDK2	IQ63_14040	Peptidase	0.17
A0A0L0JM37	IQ63_36380	D-alanyl-D-alanine dipeptidase	0.20
A0A0L0KPT5	IQ63_02400	Peptidase M4	0.03
A0A0L0JFK5	IQ63_42705	Peptidase	0.06
A0A0L0JRI4	IQ63_33665	Serine protease	0.20
A0A0L0K4M0	IQ63_22620	Glutamate-binding protein	0.18
A0A0L0KK54	IQ63_06295	Amidase	0.30
A0A0L0KP27	IQ63_01295	Peptidase S8	0.06
A0A0L0KPZ9	IQ63_01380	Streptogrisin	0.50
A0A0L0JVA0	IQ63_31405	Peptidase M48	0.54
A0A0L0JC63	IQ63_44705	Peptidase M15	0.76
A0A0L0JFB2	IQ63_43000	Serine protease	0.64
A0A0L0JWH3	IQ63_30550	Peptidase M28	0.29

A0A0L0JH72	IQ63_41570	X-prolyl-dipeptidyl aminopeptidase	0.62
A0A0L0K6H7	IQ63_19895	Gamma-glutamyltransferase	1.26
A0A0L0KC74	IQ63_14030	Serine protease	0.38
A0A0L0K833	IQ63_18455	Peptide ABC transporter substrate-binding protein	2.41
A0A0L0K519	IQ63_21405	Glycine/betaine ABC transporter substrate-binding protein	0.15
A0A0L0KPH8	IQ63_02765	ABC transporter substrate-binding protein	1.18
A0A0L0KEL2	IQ63_13240	Tripeptidyl aminopeptidase	0.58
Lipid transport and metabolism			
A0A0L0KIC3	IQ63_09235	Triacylglycerol lipase	0.13
A0A0L0KKU2	IQ63_07390	Glycerophosphodiester phosphodiesterase	0.13
A0A0L0K4E8	IQ63_21355	Cholesterol esterase	0.09
A0A0L0JN14	IQ63_35530	Glycerophosphodiester phosphodiesterase	0.19
A0A0L0KH73	IQ63_09765	Lipase	1.51
A0A0L0JYV2	IQ63_28155	Phosphoric diester hydrolase	0.17
Energy production and conversion			
A0A0L0KB99	IQ63_14755	ATP synthase subunit b	0.70
A0A0L0JH14	IQ63_41620	Oxidoreductase	0.38
A0A0L0JTB4	IQ63_32135	FAD-binding dehydrogenase	0.34

A0A0L0KK62	IQ63_06355	Cytochrome C oxidase subunit II	0.56
A0A0L0JJE9	IQ63_40360	Oxidoreductase	0.35
Cell envelope biogenesis			
A0A0L0K5M5	IQ63_21010	Cell wall anchor protein	0.18
A0A0L0JN60	IQ63_35560	Lipoprotein	0.70
A0A0L0KA05	IQ63_16180	D-alanyl-D-alanine carboxypeptidase	0.94
A0A0L0KC17	IQ63_14630	Translocation protein TolB	0.41
A0A0L0K713	IQ63_17995	Murein L,D-transpeptidase	0.30
A0A0L0JMS5	IQ63_35840	Translocase	0.39
Replication, transcription and translation			
A0A0L0JHR0	IQ63_41665	Transcriptional regulator	0.21
Nucleotide transport and metabolism			
A0A0L0JLU2	IQ63_37355	5'-nucleotidase	0.32
A0A0L0KLX8	IQ63_07075	Nucleotidase	0.51
A0A0L0K2K4	IQ63_24945	ABC transporter substrate-binding protein	0.14
A0A0L0JF31	IQ63_42895	Endonuclease/exonuclease	0.09
Inorganic ion transport and metabolism			
A0A0L0KLJ8	IQ63_06790	Alkaline phosphatase	0.20
A0A0L0KDK0	IQ63_12920	Sulfonate ABC transporter ATP-binding protein	0.04
A0A0L0K3T9	IQ63_23855	Phytase	0.27

A0A0L0JMV9	IQ63_35715	ABC transporter substrate-binding protein	0.04
A0A0L0JL47	IQ63_37275	Phosphate-binding protein PstS	0.32
A0A0L0JKR4	IQ63_38875	Iron siderophore-binding protein	0.18
A0A0L0KLT1	IQ63_04860	Phytase	0.15
Defense mechanism and stress response			
A0A0L0JDA4	IQ63_44820	β-lactamase	0.08
A0A0L0JH66	IQ63_42215	Glutathione-binding protein GsiB	0.20
A0A0L0KMV6	IQ63_03040	Glutathione-binding protein GsiB	0.45
A0A0L0JQU4	IQ63_34920	Superoxide dismutase	0.32
Coenzyme transport and metabolism			
A0A0L0JET5	IQ63_43270	Cobalamin biosynthesis protein CobN	0.34
General function prediction only			
A0A0L0KN67	IQ63_04105	Penicillin amidase	0.28
A0A0L0K2X3	IQ63_24505	Galactose oxidase	0.09
A0A0L0K7P3	IQ63_18095	Phosphoesterase	0.10
A0A0L0KNM3	IQ63_04955	ABC transporter substrate-binding protein	0.09
A0A0L0K2P1	IQ63_24950	ABC transporter substrate-binding protein	0.30
A0A0L0JS16	IQ63_34000	ABC transporter substrate-binding protein	0.10
A0A0L0JWR8	IQ63_29510	ABC transporter substrate-binding protein	0.32
A0A0L0JWG6	IQ63_30525	Pyrrolidone-carboxylate peptidase	0.60

A0A0L0KGX3	IQ63_11340	Lysyl oxidase	0.24
Function unknown			
A0A0L0JMQ0	IQ63_35735		0.45
A0A0L0JKU4	IQ63_37810		0.35
A0A0L0K4H8	IQ63_21540		0.56
A0A0L0K138	IQ63_26010		0.65
A0A0L0K3A7	IQ63_23310		0.19
A0A0L0JKG8	IQ63_39855		0.95
A0A0L0JEL8	IQ63_43540		0.51
A0A0L0KMW3	IQ63_04865		0.59
A0A0L0KQ47	IQ63_01270		0.25
A0A0L0JHC4	IQ63_41400		0.48
A0A0L0KFM7	IQ63_11780		0.37
A0A0L0KJ41	IQ63_09360		0.15
A0A0L0JYZ0	IQ63_28645		0.11
A0A0L0JES8	IQ63_43210		0.34
A0A0L0JCG1	IQ63_44650		0.69
A0A0L0K8N1	IQ63_16960		0.08
A0A0L0JWX4	IQ63_30290		0.16
A0A0L0K2Q4	IQ63_25110		0.32

A0A0L0KFN3	IQ63_11715	0.20
A0A0L0JTW9	IQ63_32370	0.06
A0A0L0JUI4	IQ63_31790	0.25
A0A0L0KQG5	IQ63_00990	0.29
A0A0L0JVZ8	IQ63_30620	0.20
A0A0L0JHW8	IQ63_41615	0.25
A0A0L0K5T7	IQ63_18930	0.21
A0A0L0JFU2	IQ63_42905	0.07
A0A0L0JKA7	IQ63_38370	0.08
A0A0L0K509	IQ63_21350	0.12
A0A0L0K5L9	IQ63_20260	0.09
A0A0L0KNB1	IQ63_03840	0.13
A0A0L0KA86	IQ63_15965	0.20
A0A0L0JD53	IQ63_45280	0.15

Table S4. Supplementary Table. Proteins produced by *S. turgidiscabies* Car8 grown in CM+suberin.

Uniprot accession number	Corresponding gene in *S. turgidiscabies* Car8 genome	Putative protein function	NSpC
Carbohydrate transport and metabolism			
L7FKW2	STRTUCAR8_05433	Glucan endo-1,3-beta-glucosidase	0.08
L7EZA5	STRTUCAR8_08888	Cellulose 1,4-beta-cellobiosidase	0.02
L7FH04	STRTUCAR8_08930	Xylose isomerase	0.07
Amino acid transport and metabolism			
L7ERR3	STRTUCAR8_09110	Zinc carboxypeptidase	0.24
L7FAE3	STRTUCAR8_03981	Peptidase families S8 and S53	0.05
L7F837	STRTUCAR8_08776	Gamma-glutamyltransferase	0.04
L7ES15	STRTUCAR8_06184	Phosphoserine aminotransferase	0.14
L7EYV6	STRTUCAR8_02802	Zinc carboxypeptidase	0.05
L7EVR4	STRTUCAR8_04164	PS-10 peptidase S37	0.16
L7FB21	STRTUCAR8_07923	4-aminobutyrate transaminase	0.90
Lipid transport and metabolism			
L7F7R8	STRTUCAR8_07544	Acetyl-CoA C-acetyltransferase	0.14
Energy production and conversion			
L7FG45	STRTUCAR8_02342	Oxidoreductase	0.21

L7EVE4	STRTUCAR8_04555	Proton-translocating NADH-quinone oxidoreductase, chain N	0.06
L7FHH6	STRTUCAR8_03044	Cytochrome c oxidase subunit 1	0.07
L7ETV2	STRTUCAR8_04876	NADH-quinone oxidoreductase subunit D	0.06
L7EVE0	STRTUCAR8_07514	ATP synthase subunit a	0.10
L7EUW7	STRTUCAR8_04568	NADH-quinone oxidoreductase subunit B	0.28
L7F7K3	STRTUCAR8_03037	Cytochrome b/b6 family protein	0.04
L7F0S1	STRTUCAR8_04989	K(+)-insensitive pyrophosphate-energized proton pump	0.05
Cell envelope biogenesis			
L7EWN1	STRTUCAR8_05621	Membrane protein insertase, YidC/Oxa1 family	0.09
Replication, transcription and translation			
L7F8N4	STRTUCAR8_04815	RNA polymerase sigma-70 factor, TIGR02952 family	0.08
L7EQC9	STRTUCAR8_04485	DNA-directed RNA polymerase subunit beta	0.09
Inorganic ion transport and metabolism			
L7EXX4	STRTUCAR8_08435	Sulfate ABC transporter	0.13
Coenzyme transport and metabolism			
L7EVM8	STRTUCAR8_02230	Pyridoxal 5'-phosphate synthase subunit PdxS	0.23
General function prediction only			

L7EVI4	STRTUCAR8_02510	Penicillin acylase	0.04
Function unknown			
L7F020	STRTUCAR8_02627		0.34
L7F9R8	STRTUCAR8_02479		0.04
L7FHP7	STRTUCAR8_07325		0.06
L7EXF9	STRTUCAR8_07161		0.15
L7FFY9	STRTUCAR8_04265		0.06

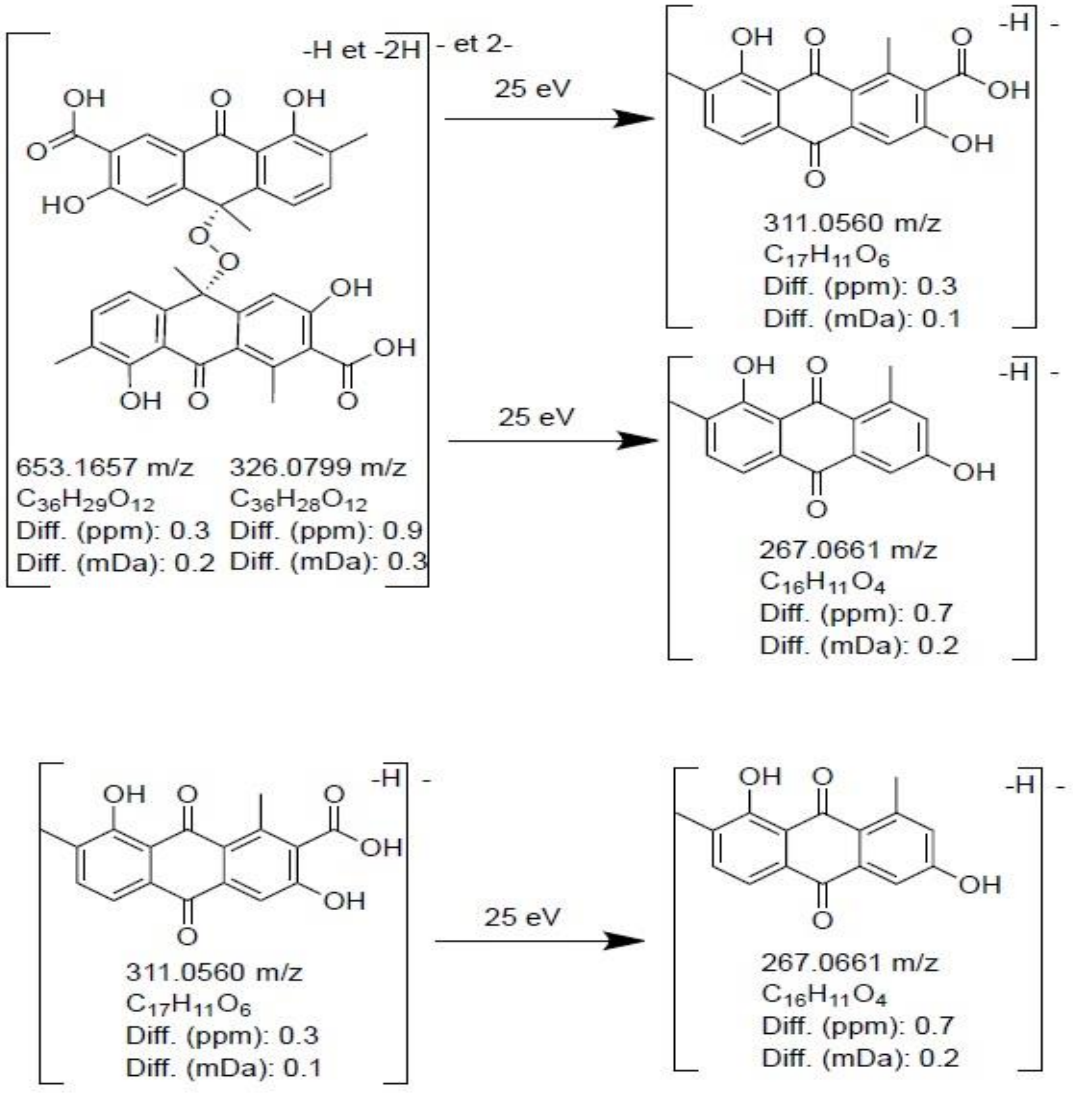

Figure F1. Supplementary figure. Fragmentation patterns of ions at 25 eV with nitrogen collision gas using a collision-induced dissociation cell.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

Suberin is a recalcitrant polymer (Rasse et al., 2005) that can persist in the soil for many years (Hamer et al., 2012). Suberin degradation is broadly attributed to fungi. However, little is known about the contribution of bacteria in this process. Evidence accumulates that the plant pathogenic bacterium *Streptomyces scabies*; the main causal agent of potato common scab produces esterases degrading the aliphatic part of suberin (Beaulieu et al., 2016). Our study identified and characterized for the first time a suberinase enzyme (sub1) in S. *scabies* which can act specifically on suberin and cutin releasing fatty acids from both substrates (Jabloune, 2018). Sub1 gene has been shown previously to be specifically overexpressed in the presence of suberin and cutin (Komeil et al., 2013). To the best of our knowledge, this is the first time that a bacterial enzyme has been shown to exhibit a suberinase activity. The production of sub1 protein by *S. scabies* in a culture medium containing suberin-enriched potato periderm was not detected until 20 days of incubation (Beaulieu et al., 2016). In the same research it was also reported that the amount of polysaccharides residues in potato periderm was markedly reduced after 5 days of incubation with *S. scabies*. These data suggest that in a minimal environment where the only source of carbon is potato suberin, *S. scabies* will meet their carbon needs first and foremost from the polysaccharide residues found in periderm.

The ability S. *scabies* to degrade the aromatic domain of suberin has not been documented. The present study is the first to investigate the ability of this bacterium to degrade the aromatic part of suberin. *S. scabies* strains were shown to efficiently utilize *trans*-ferulic and *p*-coumaric acids which are the two main constituents of

suberin aromatic part through β-ketoadipate pathway. This pathway is known for its role in the degradation of aromatic compounds derived from lignin and other plant compounds that are recalcitrant or resistant to degradation. Through this pathway, the aromatic compounds protocatechuate and catechol are converted to acetyl coenzyme A and succinyl coenzyme A. *S. scabies* was also shown to retrieve ferulic acid from suberin-enriched potato periderm and utilize it as a carbon source. Previous research showed the importance of *trans*-ferulic acid in linking the two suberin domains together and keeping the integrity of both suberin and periderm wax therefore preventing the pathogens entry (Serra et al., 2010). The ability of *S. scabies* to utilize the ferulic acid contained in potato periderm could facilitate the entry of this bacterium by disassociating the two suberin domains. The presence of phenolic compounds in plants is known to confer resistance either directly through their toxicity (De Vecchi and Matta, 1989) or indirectly through activation of post infection responses in the hosts (Harborne, 1988). The resistance of the potato cultivars against *S. scabies* was shown to be directly correlated with occurrence of phenolic acids in the peels of the potato cultivars (Singhai et al., 2011). The occurrence of the phenolic acids in the peels of the susceptible cultivars was either low or absent and scab incidence in those cultivars was very high (Singhai et al., 2011). It was also observed that peels of resistant cultivars possessed high amount of hydroxycinnamates such as ferulic and coumaric acids (Singhai et al., 2011). Moreover, the phenolic compounds synthesis was found to be involved in the durable resistance of potato to another pathogen; *Phytophthora infestans* which is the causal agent of potato late blight (Hao et al., 2018).

S. scabies could be protected from the toxic effect of these hydroxycinnamates produced by plants by its ability to efficiently degrade them. In contrast, both *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 did not show the same degradation ability as *S. scabies* 87.22 to these compounds. The degradation of

aromatic compounds via the β-ketoadipate pathway had been shown also to be necessary for the pathogenicity of other plant pathogens such as *Fusarium oxysporum* (Michielse et al., 2012).

Both *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 are emerging common scab-inducing species that became pathogenic by the acquisition of the pathogenicity island (PAI) from the original strains (Kers et al., 2005). However, it is notable that the genetic background of strains acquiring the pathogenicity island (PAI) influences the ability of the recipients to produce thaxtomin A and cause infection. This explains why some species do not become pathogenic even after acquiring PAI (Zhang et al., 2018). In addition to PAI, both *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 have the genetics required to become pathogenic however, both strains were not as well adapted as *S. scabies* 87.22 to potato tuber and to suberin. *S. scabies* 87.22 was shown to grow better on potato periderm than the other scab-causing species and even produce a suberinase similar to the cutinase CcCUT1 of the fungus *Coprinopsis cinerea* (Komeil et al., 2013). The suberinase gene (*sub1*) was detected only in *S. scabies* and was not found in *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 genomes (Komeil et al., 2013).

Many plant-pathogenic and saprophytic fungi and bacteria produce enzymes responsible for plant cell wall degradation. These polysaccharide-degrading enzymes function as a virulence factor in microbial plant pathogens (Ochiai et al., 2007). In this study both *S. scabies* 87.22 and *S. acidiscabies* ATCC 49003 showed ability to degrade the cell wall polysaccharides which indicates that both strains are genetically adapted to degrade plant cell walls. During the infection of the tuber, the bacterium first meets the periderm of the potato that contains suberin. Thus, the regulation of cellulolytic enzymes by suberin is an adaptation that *S. scabies* 87.22 has developed

to better colonize its host and degrade the cellulose present in the cell wall of the potato periderm. In addition, the biosynthesis of cellulolytic enzymes induced by suberin will allow the bacterium to obtain cellobiose, which in combination with suberin will increase the production of thaxtomin A which is essential for the development of potato common scab. On the other hand, cellulases of *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 did not show the same adaptation as *S. scabies* 87.22 since their corresponding genes were not induced by suberin.

S. scabies 87.22 was also adapted to the aromatic part of suberin. The suberin aromatic domain is a lignin-like structure (Bernards and Lewis, 1998; Bernards et al., 1995). The biological degradation of lignin is enzymatically difficult to accomplish and obviously a specific feature of filamentous fungi found in the phyla of Basidiomycota and Ascomycota (Liers et al., 2011). The ability of *S. scabies* 87.22 to efficiently degrade the two main components of suberin aromatic domain (*trans*-ferulic and *p*-coumaric acids) was not seen with the emergent pathogens *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8.

The ability of *S. scabies* to degrade the aliphatic part of suberin along with the aromatic part may give it an ecological advantage that allows it to be maintained over long periods of time in agricultural soils especially that *S. scabies* was reported to be able to survive for many years in the soil, even in the absence of its host (Ensign, 1978). Moreover, these data suggest that suberin-rich tissues can represent an ecological niche for *S. scabies*.

The obtained results provide new insights into understanding the mechanisms of adaptation of *S. scabies* 87.22 to the potato periderm. These mechanisms could

participate in the ecological success of *S. scabies* 87.22 to better colonize its host plant and decrease the ability of the emerging pathogens being able to sustain themselves in soil over long periods and therefore, affecting their capacity to infect potato tubers. These data also deepen the fundamental understanding of the biology of common scab-causing pathogens which could help in developing more effective disease control techniques.

Further research is required to determine the involvement of the *sub1* gene and the degradation of hydroxycinnamates through the β-ketoadipate pathway in the pathogenicity of *S. scabies*. It could be also interesting to identify the accessory proteins that may be actively involved in the breakdown of suberin architecture. That is the case for two feruloyl esterases (C9ZE96 and C9YVP7) that were detected in the secretome of *S. scabies* in the presence of suberin and their corresponding genes were found to be overexpressed in control media supplemented with suberin (Komeil et al., 2014). Feruloyl esterases may possibly disassociate the two suberin domains, making the substrate more accessible to hydrophilic enzymes. The future work will be also extended to determine and compare the populations of *S. scabies* 87.22, *S. acidiscabies* ATCC 49003 and *S. turgidiscabies* Car8 in soil and root samples and to monitor the transcriptomic changes of the three strains during potato tuber colonization. Developing new potato cultivars with improved content in phenolic acids could be also useful to generate disease resistant varieties through conventional breeding techniques and genetic engineering.

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